Hospital Library

VOLUME LXXIV NUMBER 6

BULLETIN of the JOHNS HOPKINS HOSPITAL

THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL
SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY

June, 1944

The Effect of Testosterone Propionate on the Arterial Blood Pressure, Kidneys, Urinary Bladder and Livers of Growing Dogs. SAM S. BLACKMAN, JR., CAROLINE BEDELL THOMAS AND JOHN EAGER HOWARD 321

Experimental Hypertension from Section of Moderator Nerves: Relationship of the Acute Pressor Response to the Development and Course of Chronic Hypertension. CAROLINE BEDELL THOMAS 335

On the Isolation and Properties of the Fluorescent Factor F_2 from Human Urine. VICTOR A. NAJJAR, VIRGINIA WHITE AND DWIGHT B. MCNAIR SCOTT 378

The Laboratory Diagnosis of Nicotinic Acid Deficiency. An Improved Procedure for the Determination of F_2 (N-Methyl Nicotinamide Derivative) in Urine. VICTOR A. NAJJAR 392

A Case of Pellagra Developing on a Hospital Ward in a Patient Receiving Vitamin B Complex. DEAN W. ROBERTS AND VICTOR A. NAJJAR 400

The Anti-Black Tongue Activity of N-Methylnicotinamide Chloride. VICTOR A. NAJJAR, MARGARET M. HAMMOND, MARY ALLEN ENGLISH, MARIA B. WOODEN AND CAROLYN C. DEAL 406

Proceedings of the Meeting of the Johns Hopkins Medical Society 415

Book Reviews 418

Index 423

PUBLISHED MONTHLY

THE JOHNS HOPKINS PRESS, Baltimore-18, Maryland

Made in United States of America

COPYRIGHT, 1944, BY THE JOHNS HOPKINS PRESS

THIS JOURNAL IS
MANUFACTURED UNDER WARTIME CONDITIONS
IN CONFORMITY WITH ALL GOVERNMENT
REGULATIONS CONTROLLING THE USE OF
PAPER AND OTHER MATERIALS

BULLETIN
OF
THE JOHNS HOPKINS HOSPITAL

MANAGING EDITOR, L. EMMETT HOLT, JR.

ASSOCIATE MANAGING EDITOR, JOHN A. LUETSCHER, JR.

EDITORIAL BOARD

E. Cowles Andrus
Charles R. Austrian
W. Halsey Barker
James Bordley, III
Chalmers L. Gemmill
Arnold R. Rich

All correspondence relating to the publication of papers should be addressed to The Editor, Bulletin of The Johns Hopkins Hospital, Baltimore-5, Md.

Books for review should be sent to The Editor at the above address.

Twenty-five reprints, without covers, of articles will be furnished to contributors free of cost. An order slip for additional reprints, with a table showing cost, is sent with proof.

Correspondence concerning business matters should be sent to The Johns Hopkins Press, Baltimore-18, Maryland, U. S. A.

This periodical is issued monthly. Two volumes a year are issued, each consisting of approximately 400 pages, and beginning in January and July, respectively.

Subscription price. In the United States and its possessions $6.00 per year. In Canada $6.25 per year; in foreign countries $6.50 per year. Prices for back volumes and back single numbers on application. Single numbers of the current volume, when available, $.75. Prices are net, postpaid.

New subscriptions and renewals are entered to begin with the first issue of the current volume. Should any issue of the current volume be out of print at the time the subscription order is received, the pro rata value of such numbers will be credited to the next volume, and the renewal invoice therefor adjusted accordingly.

Subscriptions should be renewed promptly. To avoid a break in your series, subscriptions should be renewed promptly. The publisher cannot guarantee to supply back issues on belated renewals.

Subscriptions, new or renewal, may be sent to the Publishers or ordered through your agent.

Subscriptions in the British Empire except Canada may be sent to: Baillière, Tindall & Cox, 8 Henrietta St., Covent Garden, London, W.C. 2.

THE JOHNS HOPKINS PRESS
BALTIMORE-18, MARYLAND, U. S. A.

Entered as second-class matter at the Baltimore, Md., Postoffice. Acceptance for mailing at special rate of postage provided for in Section 1103, act of October 3, 1917. Authorized on July 3, 1918.

THE EFFECT OF TESTOSTERONE PROPIONATE ON THE ARTERIAL BLOOD PRESSURE, KIDNEYS, URINARY BLADDER AND LIVERS OF GROWING DOGS[1]

SAM S. BLACKMAN, JR., CAROLINE BEDELL THOMAS, AND JOHN EAGER HOWARD

From the Departments of Pathology and Medicine of the Johns Hopkins University

The effects of androgens on the blood pressure are not well known and there are conflicting reports in the literature concerning the action of testosterone propionate on the arterial blood pressure of man and experimental animals. Grollman, Harrison and Williams reported that testosterone propionate produced systolic hypertension in rats (1). Welsh, Rosenthal, Duncan, and Taylor found the drug without effect on the systolic blood pressure of young female dogs (2). In Lattimer's experiments the systolic pressures of unilaterally nephrectomized rats and the pressure of intact and unilaterally nephrectomized dogs were not significantly altered by treatment with testosterone propionate (3). Di Grazia reported a fall in the mean arterial blood pressure of dogs following intraperitoneal injections of testosterone (4).

Steinach (5), Walker (6), Marquez (7), and Lesser (8) have reported that testosterone propionate may reduce the blood pressure of patients with arterial hypertension. Green (9) and Lattimer (3) found the drug without definite effect on the blood pressure of their patients.

In view of these divergent opinions, and because the blood pressure in most experiments previously reported was measured by indirect methods, the following observations on direct measurements of arterial pressure in growing dogs are described.

The effects of the androgen on the development of the puppies, on their livers, kidneys, and on other tissues were also studied. The results were for the most part in general agreement with those recorded in the literature. Unexpected, however, were the effects observed on

Supported in part by the Commonwealth Fund for the Study of Essential Hypertension.

the liver, and the even more striking hypertrophy of the urinary bladder, a change not hitherto described.

EXPERIMENTAL PROCEDURE

The experiments were carried out on a litter of seven mongrel puppies, three and a half weeks old at the beginning of the experiment, at which time the dogs varied in weight from 720 to 1135 grams. Four of the animals received subcutaneous injections of testosterone propionate in oil (Perandren[2]). The remaining three served as controls.

All animals were fed an identical diet throughout the experiment, consisting chiefly of whole milk and scrambled egg yolk. The amount of food eaten by each dog was not measured. The animals all appeared to thrive.

Two males (dogs 32 and 36) were observed for a period of six weeks, and were then killed (intravenous ether) and autopsied 24 hours after the treated animal had received the last injection of Perandren. During this experiment one animal (dog 32) received a total of 420 mg. of testosterone propionate, given in three 25 mg. doses each week for 4 weeks, two 25 mg. doses and one of 10 mg. the 5th week, and six 10 mg. doses during the 6th week. Dog 36 served as control and received no injections. The blood pressure of these two dogs was not measured.

A second group of dogs was observed for a period of 14 weeks; the animals were then killed (intravenous ether) and autopsied 24 to 48 hours after the treated dogs had received the last injections of Perandren. During the 14 weeks each of three animals (1 male and 2 females) received a total of 970 mg. of testosterone propionate, and two untreated dogs (1 male and 1 female) were controls. The treated dogs were given 50 to 75 mg. of testosterone propionate in divided doses each week. The number of weekly injections varied from two to six.

The organs of all the dogs were washed and weighed at autopsy. Blocks of tissue were fixed in Helly's fluid. Microscopical sections were stained with hematoxylin and eosin.

[2] This material was furnished through the courtesy of Ciba Pharmaceutical Products, Inc.

RESULTS

Arterial Blood Pressure

Observations were made to determine the effect of testosterone propionate on the arterial blood pressure of three treated animals and two controls. Between November 22 and December 12, 1940, beginning approximately ten weeks after the injections of testosterone propionate were started, pressures were recorded on each puppy on four occasions at intervals of from 4 to 7 days. Direct arterial pressures were taken by the method of Hamilton (10). The femoral artery was punctured with a 22-gauge needle without anesthesia. The puppies had been

TABLE 1

Arterial Blood Pressure of 3 Dogs Treated with Testosterone Propionate and of 2 Litter Male Controls

DOG	DIRECT ARTERIAL PRESSURES IN MM. OF MERCURY							
	Nov. 22		Nov. 26		Dec. 3		Dec. 10	
	Systolic	Diastolic	Systolic	Diastolic	Systolic	Diastolic	Systolic	Diastolic
35—Male, Control	146	73	167	98	169	87	168	93
31—Male, Treated	126	65	153	88	155	93	153	95
37—Female, Control	152	85	150	84	163	95	171	103
34—Female, Treated	154	91	140	82	143	83	150	92
33—Female, Treated	146	90	169	107	160	95	152	86

trained to lie quietly while they were gently held in place during the procedure. The arteries were readily punctured and there was no struggling.

The arterial pressures obtained were fairly stable from day to day. The pressures of the treated group did not change progressively as the treatment continued. There was no great difference in the pressures of the treated and control animals. The mean arterial pressure of the treated group was 150/89 mm. of mercury, while that of the control group was 161/90 mm. Neither the systolic nor diastolic pressure of any animal was consistently higher or lower than the rest.

The systolic pressures of the treated dogs were generally a little lower than those of the corresponding controls of the same sex. Of

the 12 systolic pressures of the treated dogs 8 were from 10 to 20 mm. lower than those of the controls on the same day, 2 were from 3 to 6 mm. lower, and 2 were higher by 2 and 19 mm. of mercury.

Of the 12 diastolic pressures of treated dogs 6 were lower by 2 to 17 mm. and 5 were higher by 2 to 23 mm. than those of the controls on the same day. On one occasion the diastolic pressures of treated dog and control were the same.

It seems justifiable to conclude that the treatment with testosterone produced no consistent change in the blood pressure of these animals.

Gross Effect on Organs

Weights of most of the organs are shown in Table 2. The data are aranged so that treated animals may be compared with controls of the same sex and period of treatment.

Each treated dog at autopsy weighed more than the corresponding control, and corresponding organs of treated and control dogs were in many instances considerably different in weight. Since the treated dogs were 200 to 300 grams heavier than the controls before treatment was started, and since the food eaten by the dogs was not measured, the significance of weight differences between the dogs and between their corresponding organs is difficult to determine, unless the differences are very marked. We have, therefore, calculated what the weights of various organs of the treated dogs should be according to the more reliable organ weight/body weight ratio, based on the formula

$$\frac{\text{Weight of organ of control}}{\text{Weight of body of control}} = \frac{X}{\text{Weight of body of treated animal}}$$

where X = the weight of the organ of the treated dog anticipated on the basis of body weight. In Table 2 the weight of each organ at autopsy is listed together with the percentage change in organ weight caused by testosterone propionate, as calculated from the above formula.

Reproductive Organs, Adrenals, and Thymus. In the treated animals marked enlargement of penis, prostate, epididymes, clitoris, vagina and uterus occurred, clearly out of proportion to the differences between the weights of the dogs. The prostates of the two treated males, for example, were 1093 and 1034 per cent heavier respectively than

TABLE 2

Effects of Testosterone Propionate on Organs of Growing Puppies

ANIMAL	DOSAGE OF DRUG	WEIGHT OF DOG (GRAMS)		WEIGHTS OF ORGANS AT AUTOPSY (GRAMS)													
		Start of exp.	At autopsy	Heart	Lungs together	Spleen	Thymus	Adrenals together	Testes together	Kidneys together	Liver	Urinary bladder	Epididymes together	Prostate	Clitoris	Vagina	Uterus	Ovaries
Treated male, 32	420 mgm. in 6 wks.	1135.0	3425.0	22.40 (+7.8%)*	Not weighed	5.52 (−13.7%)	6.15 (−65.0%)	0.37 (−24.4%)	0.10 (−73.6%)	28.00 (−0.80%)	154.0 (+17.1%)	2.90 (+16.3%)	0.75 (+120.5%)	5.25 (+1093.0%)				
Control male, 36	None	820.0	3100.0	18.82	Not weighed	5.80	15.95	0.45	0.35	25.56	119.11	2.28	0.31	0.40				
Treated male, 31	970 mgm. in 14 wks.	1100.0	8618.4	46.90 (+5.1%)	110.5 (+12.1%)	14.05 (+10.1%)	20.20 (−29.3%)	0.78 (−29.0%)	0.27 (−40.0%)	62.20 (+12.3%)	343.2 (+14.1%)	5.45 (+33.2%)	1.50 (+112.6%)	11.80 (+1034.0%)				
Control male, 35	None	825.0	6622.5	33.00	75.75	9.80	22.00	0.85	1.30	42.55	231.1	3.15	0.55	0.80				
Treated female, 34	970 mgm. in 14 wks.	800.0	7257.6	48.50 (+1.2%)	121.3 (+4.2%)	16.00 (+6.95%)	17.40 (−44.9%)	0.65 (−45.8%)		62.05 (+17.2%)	340.20 (+15.8%)	4.40 (+46.6%)			4.00 (+166.6%)	4.20 (+86.6%)	2.85 (+72.7%)	0.25 (−58.3%)
Treated female, 33	970 mgm. in 14 wks.	960.0	5783.4	35.45 (−7.1%)	98.2 (+7.8%)	10.30 (−15.0%)	7.20 (−71.3%)	0.55 (−42.1%)		47.10 (+11.1%)	276.40 (+17.9%)	4.70 (+96.6%)			3.25 (+173.1%)	7.23 (+303.9%)	2.20 (+67.9%)	0.25 (−46.8%)
Control female, 37	None	720.0	4825.0	31.85	76.00	9.95	21.00	0.80		35.20	195.45	2.00			1.00	1.50	1.10	0.40

* Figures in parentheses represent percentage change in organ weight caused by testosterone propionate, calculated from the expression $\frac{A-E}{E}$ when A represents organ weight of the treated animal and E the expected organ weight of the treated animal, based on organ weight/body weight ratio of the untreated control.

their calculated expected weights; the epididymes 120.5 and 112.6 per cent heavier. The two treated males weighed only 10.4 and 30.1 per cent more than the corresponding controls. In the two treated females enlargement of vagina, uterus, and clitoris, compared with corresponding organs of the control, varied from 67.9 to 303 per cent.

The adrenals, testes, ovaries, and thymus of the treated dogs weighed less than those of the controls, although the treated animals themselves weighed more.

Bladder, Kidneys and Liver. The urinary bladders of the treated dogs weighed from 27.1 to 135 per cent more than those of the controls of corresponding sex and duration of treatment. The weights of the bladders of the treated dogs were from 16.3 to 96.6 per cent greater than their calculated expected weights based on organ weight/body weight ratios. The most marked enlargement occurred in the bladders of the two females treated for 14 weeks. At autopsy there was no evidence of obstruction or infection of the urinary tracts. The bladders were contracted. The ureters and kidney pelves were not dilated.

The kidneys of the treated dogs weighed from 9.5 to 76.2 per cent more than those of corresponding controls of the same sex. Based on the organ weight/body weight ratios the weights of the kidneys of the dogs treated for 14 weeks were from 11.1 to 17.2 per cent more than the expected calculated weights, while the kidneys of the dog treated for only six weeks weighed almost exactly the same as the calculated expected weight.

The livers of the treated dogs weighed from 29.2 to 74.3 per cent more than those of the corresponding controls of the same sex, and they weighed 14.1 to 17.9 per cent more than their calculated expected weight.

Heart, Lungs and Spleen. The autopsy weights of the heart, lungs and spleen, and the percentage difference between the actual and the calculated weights, based on organ weight/body weight ratio are given in Table 2. The differences are small (-15% less to $+12.1\%$), and are of doubtful significance.

Microscopical Changes

Microscopical sections were made of the hypophysis, salivary glands, lymph nodes, thyroid, thymus, voluntary muscles, rib, aorta, heart,

lungs, stomach, intestine, liver, spleen, pancreas, kidneys, adrenals, bladder, penis, prostate, epididymis, clitoris, vagina, uterus, and ovaries.

It is well known that in animals certain organs, such as the genitalia, become enlarged following injections of androgens, and that others are reduced in size (ovaries, testes, adrenals, and thymus). The histological changes which accompany these alterations in size were observed by us, but since they have been well described in the literature, this need not be repeated here.

Kidneys. There were no microscopical lesions in the kidneys of any of the dogs. The blood vessels, glomeruli and tubules were histo-

TABLE 3

Average Diameter of 20 Convoluted Tubules near Boundary Zone of Kidneys of Dogs Treated with Testosterone Propionate and of Controls

DOG	DOSE	AVERAGE DIAMETER OF 20 TUBULES IN MICRONS
32—Male, Treated	420 mg. in 6 wks.	40.4
36—Male, Control	none	36.4
31—Male, Treated	970 mg. in 14 wks.	51.0
35—Male, Control	none	44.0
33—Female, Treated	970 mg. in 14 wks.	49.2
34—Female, Treated	970 mg. in 14 wks.	60.4
37—Female, Control	none	35.8

logically normal. No differences could be detected between the appearance and size of the juxtaglomerular (Goormaghtigh) bodies of the treated and corresponding control animals.

The diameters of 20 convoluted tubules were measured in one kidney of each dog. Convoluted tubules were selected for measurements where they lie in groups parallel to each other near the boundary zone. The average diameter of the tubules of the dogs treated for 14 weeks was somewhat greater than that of the controls. There was little difference between the average diameter of the tubules of the dog treated for six weeks and that of his control (Table 3).

Urinary Bladders. None of the bladders showed any evidence of cystitis. The cellular character and thickness of the mucosa and

submucosa seemed to be identical in the bladders of the treated and untreated dogs. Attempts to measure the thickness of the smooth muscle fibers were unsuccessful.

Livers. Microscopically none of the livers appeared to contain visible droplets of fat in the cells, judging by the absence of vacuoles in the hematoxylin and eosin preparations. The cytoplasm of the liver cells of each of the dogs had the coarse granular appearance generally associated with glycogen deposits, but the glycogen content was not measured. No histological difference was detected to account for the increase in weight of the livers of the treated dogs.

COMMENT

Testosterone propionate under the conditions of this experiment did not cause either arterial hypertension in the puppies, or lesions in their kidneys. The systolic blood pressure of the treated dogs was in general slightly lower than that of the corresponding controls on the same day. The results of these experiments are similar to those of Welsh, Rosenthal, Duncan and Taylor (2), Lattimer (3), and Di Grazia (4), and are contrary to those of Grollman, Harrison and Williams (1).

Welsh, Rosenthal, Duncan, and Taylor, in their experiments with 3 young female dogs, gave 100 mgm. daily subcutaneous injections of testosterone propionate for periods ranging from 6 to 21 days. The systolic blood pressure was measured indirectly by palpation, using a small cuff on the hind leg. The pressure was measured "at intervals" during a control period, while testosterone was being administered, and during the recovery period. The drug had no observed effect on the systolic pressure.

In Lattimer's experiments 3 intact and 2 unilaterally nephrectomized adult bitches were injected daily for 28 days with 10 mg. doses of testosterone propionate. Systolic pressures were measured by palpation of carotid loops. No significant variations in pressure were found. Twenty-three unilaterally nephrectomized rats were given daily injections of testosterone propionate. The dosage varied from 1 mg. daily for 35 days to 5 mg. daily for 10 days. Systolic pressures were measured every 4 days by the method of Grollman, Harrison, and Williams. The pressure of one rat rose from 130 to 160 mm. of mercury

following injections of the drug. The pressures of the other rats did not vary significantly after treatment.

Di Grazia found that in dogs 1 mg. of testosterone per kg. of body weight when injected intraperitoneally caused a drop in mean arterial pressure which amounted to as much as 30 mm. of mercury in some animals, began 10–15 minutes after injection and reached a maximum in 60 to 80 minutes.

Grollman, Harrison, and Williams reported that testosterone propionate caused hypertension in rats. In their experiments the dosage of testosterone propionate used, the duration of treatment, and the degree of hypertension produced were not specified. Blood pressure curves were presented to show the effect of desoxycorticosterone and estradiol benzoate; and the effect of testosterone propionate was said to be qualitatively similar to that of desoxycorticosterone. The hypertension was thought by them to be due to renal damage, although the kidneys were not examined.

The relatively very great enlargement of external genitalia, prostate, epididymes, vagina, and uterus, and the comparatively small size of the adrenals, thymus, ovaries and testes observed in the growing dogs injected with testosterone propionate are in the nature of specific effects. They occur in other species of animals treated with androgens, and have been repeatedly described (11, 12, 13, 14, 15, 16, 17, 18, 19).

Enlargement of the kidneys following the use of androgens has been seen in a number of species including the rabbit (20), mouse (21, 22), rat (23, 24, 25, 26, 27, 28) and dog (3). It occurs in both sexes and is described as being due to hypertrophy of the renal tubules in the rat (25, 27) and dog (3). In mice, in addition, there is hypertrophy of Bowman's parietal glomerular epithelium (29, 21).

Renal enlargement is said to be more obvious in the female than in the male (17), to be more pronounced in the mouse than in the rat (17), to persist for at least a month in the rat after discontinuance of the drug (26), and is not prevented by hypophysectomy (30). The degree of renal hypertrophy which follows unilateral nephrectomy is increased by testosterone propionate in the rat (31, 3, 28) and dog (3). Testosterone propionate is a more effective renotropic androgen than allied steroids (22). It has been suggested that androgens may be of use in some cases of Bright's disease on account of their renotropic

action (3, 25) and it has been found that renal inulin clearance and diodrast Tm are increased by the administration of testosterone propionate to dogs (2, 3).

The literature on the renotropic effect of androgens is plentiful, and from it one gets the impression that these substances affect the kidneys more than organs such as the heart, lungs, and liver. Concerning this point, however, there is no close agreement, and in most of the papers dealing with the effect of androgens on the kidneys of intact animals relatively little can be found concerning the weights of other organs.

Korenchevsky, Hall, and Burbank, however, reported that male hormone, including testosterone propionate, causes hypertrophy of the liver, kidneys, heart, and spleen of female rats (32). Korenchevsky and Hall found no effect on the thyroid, spleen, and heart of adult and senile male rats, although there was a 12 per cent increase in the weight of the kidneys (33). Selye gave testosterone to albino rats 7 weeks old for 21 days (17). The average weight of the hearts of six rats was slightly increased in the males, decreased in the females; and the spleen was slightly increased in weight in the females, decreased in the males compared with the controls. Selye made incidental mention of finding marked increase in the size of the pancreas of normal and hypophysectomized rats given testosterone (30). In an earlier paper Selye tabulated the average weights of kidneys, liver, and spleen of 7 adult female mice treated with large doses of testosterone (5 mg. daily for 20 days) (34). The organs were weighed after being fixed. The average weight of the kidneys of the treated mice was 0.1371 grams more than that of the controls. The average weight of the livers was 0.123 grams less than that of the controls. The administration of male sex hormone to castrated rats is said to cause hypertrophy of the liver, heart, kidney, and spleen (35, 36). Kochakian implanted pellets of testosterone propionate in the subcutaneous tissues of castrated male mice, and later found marked enlargement of the kidneys, together with slight enlargement of the hearts and livers (37).

In this experiment with puppies the data indicate that the livers of the treated dogs and the kidneys of those treated for 14 weeks were enlarged to about the same degree (11 to 17 per cent, based on organ weight/body weight ratios). These results suggest that in the growing dogs the effect of the hormone on the liver was as marked as the action

on the kidneys, and that the increased weight of both liver and kidneys was not greatly out of proportion to the general body development. It is interesting in this connection, however, that the hearts of the treated dogs did not appear to participate in the accelerated growth.

The bladder of each treated dog was heavier than that of the corresponding control, and the degree of enlargement was considerable, especially in the dogs treated for 14 weeks. Since we were unable to measure the thickness of the smooth muscle fibers microscopically, and since the majority of tissue in the wall of the bladder consists of muscle, we have assumed rather than demonstrated that the muscular walls of the bladders were hypertrophied.

If this assumption is correct there are apparently two possible explanations. The urethra may have been partially obstructed by the enlarged prostate in the males and by the hypertrophied clitoris in the females. Against this explanation, however, is the complete absence of cystitis, and the absence of any dilatation of the ureters.

The other possible explanation is that the hormone caused hypertrophy of the smooth muscle of the bladder. This seems probable in view of the fact that it induces hypertrophy of other smooth muscle (uterus and corpus cavernosum) and of voluntary muscles (38, 39).

CONCLUSIONS

Testosterone propionate given subcutaneously to growing puppies of both sexes for periods of 6 and 14 weeks produced effects which are well known in other species of animals. The androgen had little effect on the arterial blood pressure of the dogs.

The liver of each treated dog and the kidneys of the dogs treated for 14 weeks were enlarged by approximately 11 to 17 per cent. The urinary bladders of the treated dogs were considerably enlarged, due probably to hypertrophy of smooth muscle. No effect of the androgen could be detected on the microscopical appearance of the renal juxtaglomerular tissue.

BIBLIOGRAPHY

1. Grollman, A., Harrison, T. R., and Williams, J. R., Jr.: The effects of various sterol derivatives on the blood pressure of the rat. J. Pharmacol. & Exp. Therap., **69**: 149, 1940.

2. WELSH, C. A., ROSENTHAL, A., DUNCAN, M. T., AND TAYLOR, H. C., JR.: The effect of testosterone propionate on renal function in the dog, as measured by the Creatinine and Diodrast Clearance and Diodrast Tm. J. Physiol., **137:** 338, 1942.
3. LATTIMER, J. C.: The action of testosterone propionate upon the kidneys of rats, dogs, and men. J. Urol., **48:** 778, 1942.
4. DI GRAZIA, A.: Azione dell'ormone orchitico sulla pressione arteriosa. Ormoni, **1:** 109, 1939.
5. STEINACH, E., PECZENIK, O., AND KUN, H.: Ueber hormonale Hyperämisierung, insbesondere über den Einfluss der männlichen Sexualharmone und ihre Kombination mit weiblichen Harmon auf erhöhten Blutdruck und Hypertonus. Wien. Klin. Wochenschr., **51:** 102, 1938.
6. WALKER, T. C.: Use of testosterone propionate and estrogenic substances in treatment of essential hypertension, angina pectoris, and peripheral vascular disease. J. Clin. Endocrinol., **2:** 560, 1942.
7. MARQUEZ, A. L.: Influence of testosterone on hypertension in men. Semana Med. (Buenos Aires), **50:** 1180, 1943.
8. LESSER, M. A.: The treatment of angina pectoris with testosterone propionate. The New Eng. J. Med., **226:** 51, 1942.
9. GREEN, R.: On the effect of testosterone propionate on the blood pressure. Lancet, **2:** 79, 1938.
10. HAMILTON, W. F., BREWER, G., AND BROTMAN, J.: Analytical description of a new high frequency hypodermic manometer with illustrative curves of simultaneous arterial intracardiac pressures. Am. J. Physiol., **107:** 427, 1934.
11. HU, C. K., AND FRAZIER, C. N.: Masculinization of adult female rabbit following injection of testosterone propionate. Proc. Soc. Exp. Biol. and Med., **42:** 820, 1939.
12. SELYE, H.: Postnatal masculinization of female rat by means of testosterone propionate. Anat. Record, **76:** 145, 1940.
13. KORENCHEVSKY, V., DENNISON, M., AND HALL, K.: Effects of testosterone and testosterone propionate on adult male rats. Biochem. J., **31:** 1434, 1937.
14. BOTTOMLEY, A. C., AND FOLLEY, S. J.: Effect of high doses of androgenic substances on weights of testes, accessory reproductive organs, and endocrine glands of growing male guinea pigs. J. Physiol., **94:** 26, 1938.
15. RUBENSTEIN, H. S., AND KURLAND, A. A.: Effect of testosterone propionate on rat testes. Endocrinol., **28:** 495, 1941.
16. SELYE, H.: Morphological changes in female mice receiving large doses of testosterone. J. Endocrinol., **1:** 208, 1939.
17. SELYE, H.: Interaction between various steroid hormones. Can. Med. Ass. J., **42:** 113, 1940.
18. LAQUEUR, G. L., AND FLUHMANN, C. F.: Effects of testosterone propionate in immature and adult female rats. Endocrinol., **30:** 93, 1942.

19. GREEN, R. R., BURRILL, M. W., AND IVY, A. C.: Masculinization of female rats by postnatal administration of male sex hormone. Proc. Soc. Exp. Biol. and Med., **38:** 4, 1938.
20. ISCOVESCO, H.: Action physiologique d'un lipöide estrait du testicule. C. R. Soc. de Biol., **75:** 445, 1913.
21. SELYE, H.: The effect of testosterone on the kidney. J. Urol., **42:** 637, 1939.
22. PFEIFFER, C. A., EMMEL, V. M., AND GARDNER, W. U.: Renal hypertrophy in mice receiving estrogens and androgens. Yale J. Biol. & Med., **12:** 493, 1940.
23. KORENCHEVSKY, V., AND DENNISON, M.: The effect on male rats of the simultaneous administration of male and female sexual hormones and the relation to the assay of the hormone. Biochem. J., **28:** 1486, 1934.
24. NATH, M. C., AND SEN GUPTA, T. N.: The action of androsterone on sexually immature male rats. Ind. J. Med. Research, **27:** 171, 1939.
25. KORENCHEVSKY, V., AND ROSS, M. A.: Kidney and sex hormones. Brit. Med. J., **1:** 645, 1940.
26. SELYE, H.: Production of persistent changes in the genital organs of immature female rats treated with testosterone. Endocrinol., **27:** 657, 1940.
27. LUDDEN, J. B., KRUEGER, E., AND WRIGHT, I. S.: Effect of testosterone propionate, estradiol benzoate, and desoxycorticosterone acetate on the kidneys of adult rats. Endocrinol., **28:** 619, 1941.
28. MACKAY, E. M.: Degree of compensatory renal hypertrophy following unilateral nephrectomy. Influence of testosterone propionate. Proc. Soc. Exp. Biol. & Med., **45:** 216, 1940.
29. CRABTREE, C. E.: The structure of Bowman's capsule in castrate and testosterone treated male mice as an index of hormonal effect on the renal cortex. Endocrinol., **29:** 197, 1941.
30. SELYE, H.: Effect of hypophysectomy on morphological appearance of the kidneys and on renotropic action of steroid hormones. J. Urol., **46:** 110, 1941.
31. KORENCHEVSKY, V., DENNISON, M., AND BROVSIN, I.: The assay and the effect of testosterone on rats compared with those of other sexual hormones. Biochem. J., **30:** 558, 1936.
32. KORENCHEVSKY, V., HALL, R., AND BURBANK, R.: The manifold effects of prolonged administration of sex hormones to female rats. Biochem. J., **33:** 372, 1939.
33. KORENCHEVSKY, V., AND HALL, K.: Prolonged injection of male sex hormone into normal and senile male rats. Brit. Med. J., **1:** 4, 1939.
34. SELYE, H.: Morphological changes in female mice receiving large doses of testosterone. J. Endocrinol., **1:** 208, 1939.
35. HALL, K., AND KORENCHEVSKY, V.: Hypertrophic effect of male sex hormone on livers of castrated rats. Brit. M. J., **1:** 438, 1938.
36. KORENCHEVSKY, V., HALL, K., AND ROSS, M. A.: Prolonged administration of sex hormones to castrated rats. Biochem. J., **33:** 213, 1939.

37. KOCHAKIAN, C. D.: Rate of absorption and effects of testosterone propionate pellets on mice. Endocrinol., **28:** 478, 1941.
38. WAINMAN, P., AND SHIPOUNOFF, G. C.: The effect of castration and testosterone propionate on the striated perineal musculature in the rat. Endocrinol., **29:** 975, 1941.
39. PAPANICOLAOU, G. N., AND FALK, E. A.: General muscular hypertrophy induced by androgenic hormone. Science, **87:** 238, 1938.

EXPERIMENTAL HYPERTENSION FROM SECTION OF MODERATOR NERVES:

Relationship of the Acute Pressor Response to the Development and Course of Chronic Hypertension[1]

CAROLINE BEDELL THOMAS

From the Department of Medicine, The Johns Hopkins University, Baltimore, Maryland

Submitted for Publication June 5, 1944

Neurogenic factors in essential hypertension have been widely recognized and described. The personality of hypertensive patients is usually characterized by increased psychomotor activity, great conscientiousness, irritability and hypersensitiveness (1, 2). The emotional pattern, it is generally agreed, is that of strongly inhibited resentment and aggressiveness constantly smoldering at the subconscious level (3, 4). The autonomic reflex responses to various stimuli are exaggerated. Hines and Brown (5) have shown that hypertensive individuals react with a greater pressor response to a standard cold stimulus than do most normal persons. Almost everyone with essential hypertension is a "hyperreactor," according to their test, and this characteristic seems, in some degree, to be an inherited trait (6). Finally, the administration of sedatives such as sodium amytal or pentothal sodium, or of spinal anesthesia, may produce a profound fall in the blood pressure of patients with essential hypertension within a few minutes (7, 8, 9). Such drugs, which primarily affect the central nervous system, show little effect upon the blood pressure of normal persons. All these considerations point to the importance of nervous mechanisms in essential hypertension.

Whether these nervous influences are of *primary* importance in the pathogenesis of essential hypertension, however, or are *secondary* manifestations of the elevated blood pressure which was produced by some mechanism entirely unrelated to the nervous system is not yet clear. Alexander (10) points out that the acute emotions of rage and fear produce temporary elevations of blood pressure, as Cannon (11) has

[1] Supported by a grant from the Commonwealth Fund for the Study of Essential Hypertension.

shown in experimental animals. He therefore believes that chronic inhibited rage may lead to a chronic elevation of the blood pressure, and that this is the basis for the early, fluctuating phase of essential hypertension before irreversible vascular changes have appeared. He also postulates a constitutional instability of the vasomotor system in hypertensive individuals. On the other hand, since Goldblatt (12) and his co-workers produced experimental hypertension by constricting the renal arteries of dogs, and it was shown that this form of hypertension is unaffected by sympathectomy, many have believed that essential hypertension in man, as well, may be of humoral origin (13, 14, 15, 16). Page and Helmer (17) have isolated angiotonin, a crystalline pressor substance derived from renin, and consider that renal artery constriction increases renin formation in the kidney. Corcoran and Page (18) call attention to the similarity of the effects of angiotonin in animals to those characterizing arterial hypertension in man, namely cardiac augmentation, arteriolar constriction and constriction of the efferent arterioles of the kidneys. They raise the possibility that angiotonin is an etiological factor in essential hypertension in man, but they recognize that there is participation of endocrine and nervous factors as well.

The height of the arterial pressure is the resultant of many pressor and depressor or moderator influences. Accordingly, all types of hypertension may be thought of as representing some disturbance in the ratio between the strain upon the moderator mechanism and its ability to cope with such strain, as was recently pointed out by Katz and Leiter (19). Either an increase in the pressor factors producing such strain, or a decrease in the ability of the moderator mechanism to lower arterial pressure would lead to the same result—maintenance of arterial pressure at a higher than normal level.

Hering (20) was the first to describe the carotid sinus nerve, and to show that the function of moderating the arterial blood pressure is shared between the two carotid sinus nerves and the two aortic nerves. He demonstrated that hypertension appears immediately after interruption of these four moderator nerves. Subsequent investigators, particularly Heymans, Bouckaert, and Regniers (21) and Bronk and Stella (22, 23), found that tonic inhibitory afferent impulses travel along the moderator nerves to the vaso-constrictor, cardio-accelerator

and adrenalino-secretory center in the medulla. When the moderator nerves are severed, these sympathetic centers are released from inhibition and develop increased activity which results in a heightened arterial pressure.

Successful attempts to produce chronic arterial hypertension by moderator nerve section were first reported in the rabbit by Hering's pupils, Koch and Mies (24), and in the dog by Koch (25) and Heymans and Bouckaert (26). They showed that if animals with severed moderator nerves are allowed to survive, chronic hypertension may develop which persists over the course of many months or years. This form of chronic experimental hypertension has been widely referred to as "neurogenic hypertension." Heymans and Bouckaert (27) have indicated that it develops acutely following nerve section and persists indefinitely. They imply that the acute and chronic forms merge into one another, and that the underlying mechanism is the same.

However, in preparing dogs with chronic hypertension from section of the moderator nerves in connection with other experiments (28, 29, 30), it has been my own experience that a latent period of a number of days may occur between the completion of the denervations and the development of the chronic hypertension, and that when the hypertension does appear, a period of time may elapse before it reaches its maximum intensity. The same observation has been briefly recorded by other investigators of this form of hypertension. Thus Kremer, Wright and Scarff (31), working with rabbits, stated that "the hypertension did not develop to its full extent till three to four weeks after the second (final) operation;" no further details were given, nor any blood pressure readings during this early period. Boyd and McCullogh (32) likewise using rabbits, reported the time in days after complete moderator nerve section when the pressure began to rise, and when it reached the maximum pressure. (See Table I.)

Nowak (33) working with dogs, noted "Marked elevation of arterial pressure was observed as early as two days after this type of denervation. Thus Dog 9, whose control pressure was 124 prior to denervation, showed an increase to 184 two days later. In Dog 7, the pressure rose from a control level of 158 to 190 on the third day. In general, the blood pressure became definitely elevated within two to three weeks after denervation." His charts show that maximum blood pressure

developed approximately three to six weeks after denervation. Likewise, Grimson's (34) composite blood pressure chart shows rising pressure levels during the first post-operative month; he reported three dogs which reached their maximum blood pressure levels four to six months after operation.

These various observations made it seem desirable to carry out more detailed studies concerning the development of the chronic form of neurogenic hypertension, with special attention to the transition period between the completion of moderator nerve section and the establishment of permanent hypertension. There were several fundamental

TABLE I

(Modified from Boyd and McCullogh)

ANIMAL	TIME IN DAYS AFTER FINAL OPERATION	
	Pressure began to rise	Pressure reached maximum
44	4	33
77	7	40
79	7	61
43	9	30
71	9	104
40	10	10
72	13	104
45	13	172
80	15	40
46	15	43
36	44	107

questions concerning this transition period which needed answering: is the initial, acute hypertension sufficiently maintained to suggest its identity with the chronic phase? Or is there a delayed, gradual development of the chronic form somewhat resembling the onset of benign renal hypertension (12)? Does the increased vasoconstriction of the renal arteries following interruption of the moderator nerves result in the increased production of renin, so that neurogenic and humoral mechanisms are finally combined? It has been demonstrated previously that the presence of renal tissue is unnecessary for the characteristic development of acute hypertension following interruption of the moderator nerves (35), but the role of the kidney in the chronic form remains to be determined.

This series of studies was therefore undertaken in the attempt to observe closely the development and clinical course of hypertension following interruption of the moderator nerves, as well as some of its physiological characteristics and the pathological changes accompanying it. The paper referred to above (35) is considered the first of the series; the present paper deals with the relationship of the acute pressor response to the chronic stage of hypertension, as well as with the clinical course of the hypertension.

METHODS

Animals and Maintenance. Twelve full grown mongrel dogs weighing from 10 to 17 kilograms were used. One male and one female developed intractable vomiting from too extensive vagotomy, and were killed. Of the ten animals which developed hypertension, nine were females and one was a male. Although apparently healthy when selected, the dogs were quarantined for a month or two before they were added to the dog colony. They were immunized against distemper and given two types of vermifuge. If mange were found, it was eradicated by repeated applications of lime-sulphur concentrate (36). The animals were housed in large wire-floored cages in a room entirely devoted to a healthy colony of dogs. The dogs usually exercised for an hour or two daily in a sheltered yard. Diet consisted of Bal-mar (later Silver Klass) Dog Food, and Purina Dog Chow on alternate days six days a week, with raw meat on the seventh day. Cod liver oil was given weekly. The quantity of food was adjusted to maintain each dog in a constant state of good nutrition. Water was given freely.

Measurement of Arterial Pressure. Most of the arterial pressure measurements were carried out by means of the optical manometer devised by Hamilton, Brewer, and Brotman (37)[2]. Using this method, the femoral artery of an unanesthetized[3] dog (trained to lie quietly on one side with paws restrained) was punctured with a 22 gauge needle. The needle was connected by a Luer lock to a length of lead tubing leading to the brass manometer; needle, tubing and manometer were filled with a 7 per cent solution of sodium citrate in physiological salt

[2] I should like to express my thanks to Dr. W. F. Hamilton for his kindness in supplying the manometer and replacement parts, with advice concerning their use.

[3] The same method was used during operations while the dog was anesthetized.

solution, from which every air bubble had been painstakingly excluded. Through this non-elastic system, oscillations in arterial pressure were transmitted directly to a flexible copper-beryllium membrane 0.008 micra in thickness mounted at one end of the manometer. Movements of the membrane were transmitted to an eccentrically mounted plano-convex mirror. An intense beam of light, projected from a 500 watt monoplane filament projection lamp and interrupted 300 times a minute by the revolving arm of a time marker, was reflected by this moving manometer mirror and a stationary baseline mirror upon the slot of a camera, so that arterial blood pressure was recorded directly upon a moving strip of sensitized paper.

The level of the manometer mirror was adjusted to the level of the site of puncture of the femoral artery. Calibration of the membrane was carried out each day before the arterial pressures were determined, and, if many pressures were taken, it was repeated at the end of the series. The same membrane was used for many months; when a new one was seated, great care was taken in selecting a membrane which could be repeatedly calibrated with accuracy.

Consistent use of this method showed it to be a highly satisfactory means of recording systolic and diastolic pressures and cardiac rate. While it demands more time and technical skill to set up and maintain the manometer in efficient running order than other methods, once it is available, pressures can be taken quickly and a permanent record is obtained. It is much the best method I have used in obtaining arterial pressures of unanesthetized dogs. (Other methods used: A. *Indirect.* 1. Sphygmomanometer of Kolls and Cash; 2. Small cuff on Van Leersum carotid loop; 3. Narrow cuff on hind leg with palpation of or auscultation over the artery. B. *Direct.* 1. Mean pressure from femoral artery puncture measured by a mercury manometer).

Before the Hamilton manometer was set up, and during the early months of its use, systolic and diastolic pressures and cardiac rate were recorded on a kymograph by the indirect method of Kolls and Cash (38, 39, 40). This is also a useful and accurate method, and the readings were fairly consistent with those obtained by the direct method of Hamilton, when the methods were used simultaneously, one on each hind leg. In three dogs[4], eight simultaneous determinations by the

[4] Dog 15: thin leg, neurogenic hypertension. Dog 27: plump leg, neurogenic hypertension. Dog 37: muscular leg, renal hypertension.

two methods were practically identical. In one dog[5], the systolic pressure was consistently lower and the diastolic higher by the direct than by the indirect method on three determinations. In another dog[6], the systolic and particularly the diastolic pressures were both consistently higher by the direct method than by the indirect method on three determinations. It seemed probable that the direct method was the more accurate of the two, and it had the great advantage of permitting continuous arterial pressures to be recorded over a period of minutes, limited only by the tendency of the blood to clot in the needle. The indirect method, on the other hand, permits only discontinuous readings at intervals of about two minutes, the diastolic level being recorded 15 or 30 seconds later than the systolic level.

A few direct mean arterial pressures appear on these charts, which were taken by puncturing the femoral artery with a 16 gauge needle attached by rubber tubing filled with sodium citrate solution to a mercury manometer.

Operative Technique. Section of the four moderator nerves under aseptic precautions was always carried out in more than one operation to avoid sudden cardiac or respiratory failure; in most animals each moderator nerve was divided at a separate operation for purposes of study. Under ether anesthesia, the carotid sinus nerve was severed, and the common, internal and external carotid arteries were stripped of their sheaths for a centimeter or two above and below the bifurcation. All smaller arterial branches arising near the bifurcation were severed. The arterial walls were then painted with a 5 per cent solution of phenol to destroy any remaining nerve fibers, and were washed with alcohol. The left vago-sympathico-depressor trunk was completely divided and a 2 cm. length removed. The medial third of the right vago-sympathico-depressor trunk was cut through, and a similar length removed. By this means the right vagus nerve was left intact, and the depressor fibers (and some of the sympathetic fibers) on that side were destroyed. This is the most difficult step in the operative procedure, to divide enough depressor fibers to produce hypertension, yet to leave enough vagal fibers intact to insure the dog's survival.

Ether was administered intratracheally, the uniformity of the concentration of ether in air being regulated by the use of an ether bottle with

[5] Dog 36: thin leg, renal hypertension.

[6] Dog 25: muscular leg, neurogenic hypertension.

damper attached to the tube. Anesthesia was maintained at a very light level, just sufficient to abolish spontaneous muscular movement, but not to abolish the corneal reflex.

Measurement of Arterial Pressure During Operation. Except in Dogs 15, 25 and 27, direct systolic and diastolic pressures and cardiac rates were recorded repeatedly throughout each operation. Control readings were made just before and just after anesthesia was induced. When the moderator nerve was completely exposed but undivided, another record was taken. The left vago-sympathico-depressor trunk was instantaneously divided during a continuous recording of arterial pressure and cardiac rate. When a carotid sinus nerve or the right depressor fibers were divided, the recording camera was stopped during the two or three minutes necessary to complete the final dissection to avoid clotting in the needle, and a new arterial puncture was carried out within 15 seconds of the completed section of the nerve. That record was continued for several minutes. One or two more records were made before the completion of the operation, and another was often obtained several hours later when the dog had regained consciousness.

Order in which the Moderator Nerves were Sectioned. In the seven dogs in which studies were carried out during anesthesia as well as in the unanesthetized state, the moderator nerves were sectioned as follows:

Dogs 43, 44 and 46
- Operation I: Right depressor fibers
- Operation II: Left carotid sinus nerve
- Operation III: Right carotid sinus nerve
- Operation IV: Left vago-sympathico-depressor trunk

Dogs 47, 48 and 49
- Operation I: Left vago-sympathico-depressor trunk
- Operation II: Right depressor fibers
- Operation III: Left carotid sinus nerve
- Operation IV: Right carotid sinus nerve

Dog 51
- Operation I: Left vago-sympathico-depressor trunk
  Right depressor fibers
  Left carotid sinus nerve
- Operation II: Right carotid sinus nerve

In the three dogs studied only in the unanesthetized state, the moderator nerves were sectioned as follows:

Dogs 15 and 25

Operation I:	Right depressor fibers
	Right carotid sinus nerve
Operation II:	Left vago-sympathico-depressor trunk
	Left carotid sinus nerve

Dog 27

Operation I:	Right depressor fibers
Operation II:	Left vago-sympathico-depressor trunk
Operation III:	Right carotid sinus nerve
Operation IV:	Left carotid sinus nerve

RESULTS

A. *The Acute Pressor Response following Moderator Nerve Section.* Acute pressor responses were observed in all the animals studied during operation. When the moderator nerves were sectioned singly, the greatest pressor response appeared immediately after section of the fourth moderator nerve in six of the seven dogs (Table II). The order of nerve section did not seem important; similar results were obtained whether the right carotid sinus nerve or the left vago-sympathico-depressor trunk were the last nerve sectioned. Following section of the first three moderator nerves pressor responses were usually slight or absent. Dogs 48 and 49 showed significant rises in pressure after section of the first nerve. Dog 43 showed moderate pressor responses following section of the last two or three nerves (see Footnote 1, Table II). Dog 46 was the only one which did not exhibit the greatest pressor response following section of the fourth nerve. In this animal, the only rise in pressure appeared following section of the third nerve. The two dogs, 44 and 51, in which the greatest rise in pressure followed section of the fourth moderator nerve, showed no significant pressor response following the three earlier operations (Figs. 1 and 2).

When nerve section produced a significant pressor response, arterial pressure and cardiac rate immediately began to rise, so that a well marked elevation was present in less than a minute. Arterial pressure reached its maximum within five minutes after nerve section, declining slowly thereafter. When observations under anesthesia were terminated, four to twenty-eight minutes (average fourteen minutes) after

TABLE II

The acute pressor response following moderator nerve section

ORDER OF MODERATOR NERVE SECTION	DOG NO.	RISE FOLLOWING OPERATION I			RISE FOLLOWING OPERATION II			RISE FOLLOWING OPERATION III			RISE FOLLOWING OPERATION IV			TOTAL RISE		
		S.P.	D.P.	C.R.	S.P.	D.P.	C.R.	S.P.	D.P.	C.R.	S.P.	D.P.	C.R.	S.P.	D.P.	C.R.
I = Right depressor fibers	43	+4	+17	+23	+33?[1]	+6?[1]	+71?[1]	+27	+17	+60	+33	+26	+5	+97?	+66?	+159?
II = Left carotid sinus nerve																
III = Right carotid sinus nerve	44	+7	+6	−	+8	+7	−	+1	+1	+4	+164	+82	+60	+180	+96	+64
IV = Left vago-sympathico-depressor trunk	46		0	−	−	−	−	+69	+38	+22	−	−	0	+69	+38	+22
I = Left vago-sympathico-depressor trunk	47			+6	+12	+9	+10	−	−	0	+29	+16	+3	+41	+25	+19
II = Right depressor fibers	48	+35	+15	+7	+3	+6	+5	0	+8	0	+40	+33	+25	+78	+62	+42
III = Left carotid sinus nerve	49	+38	+15	+7	+6	0	−	+8	+15	+20	+61	+54	+67	+113	+84	+94
IV = Right carotid sinus nerve	51	+3	+6	−	−	+7	+3	+6	+6	+15	+90	+43	+37	+99	+62	+55

S.P. = Systolic pressure in mm. Hg; D.P. = diastolic pressure in mm. Hg; C.R. = cardiac rate per minute.
− = Slight fall. [1] = Rise followed exposure of nerve, before section; nerve traumatized?

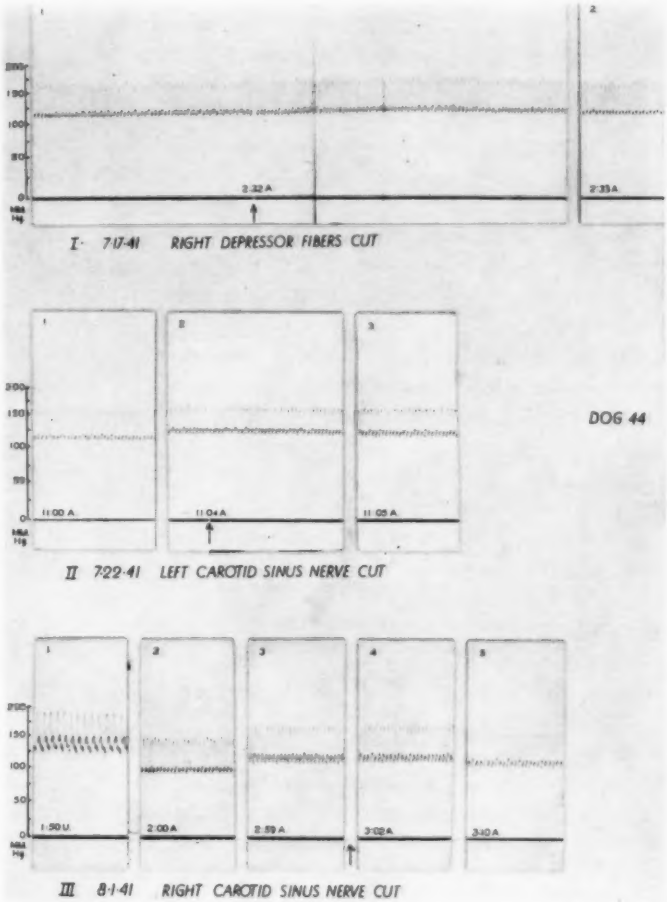


FIG. 1. DOG 44

Records taken during the three operations at which the first three moderator nerves were sectioned. Arrows indicate the moment of nerve section. U. = unanesthetized. A. = anesthetized. In none of these three operations did nerve section produce any alteration in arterial pressure or cardiac rate.

nerve section, appreciable elevation of arterial pressure was still present in all but two animals. In Dog 46, arterial pressure had returned to control levels eighteen minutes after section of the third moderator

nerve, and in Dog 47, four minutes after section of the fourth moderator nerve.

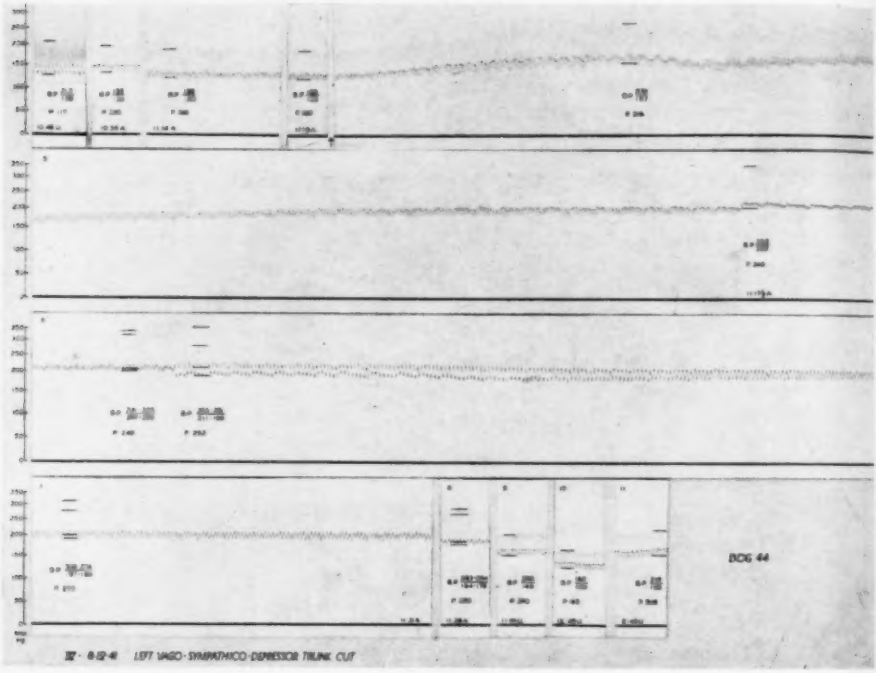


FIG. 2. DOG 44

The fourth moderator nerve, in this case the left vago-sympathico-depressor trunk, was severed at the point indicated by the arrow. Arterial pressure and cardiac rate began to rise immediately, reaching a maximum 2½ and 3 minutes later respectively. Pulsus alternans then developed, and the arterial pressure began to decline. Following the operation arterial pressure had returned to normal levels but the cardiac rate was still elevated.

B. P. = blood pressure; P. = pulse (cardiac rate); U. = unanesthetized; A. = anesthetized.

The most striking acute pressor response, seen in Dog 44, is illustrated in Figure 2. In this animal arterial pressure and cardiac rate rose continuously for 2½ minutes. During the next thirty seconds there

was very little further rise, but at the end of that time pulsus alternans developed, and a half minute later, the arterial pressure started to fall very gradually. This decline in pressure was still continuing thirteen minutes after nerve section, at which time anesthesia was discontinued. During the next hour, with the dog unanesthetized, pressure fell below the control level, but at the end of three hours systolic pressure had returned to the control level, diastolic pressure was somewhat higher, and cardiac rate very much higher than the control level.

B. *The Interval between the Acute Pressor Response and the Development of Chronic Hypertension.* There is no evidence to show that the arterial pressure of Dog 51 ever returned to normal following section of the fourth moderator nerve. Average pressure was 192/118 in the three determinations preceding the fourth operation. Fifteen minutes after the nerve was cut, with the dog still anesthetized the pressure was 278/168, and three hours after recovery from anesthesia it was 269/162. Twenty-four hours after operation the arterial pressure was 348/159.

In the other dogs, arterial pressure had returned to normal either before the end of the operation (Dogs 46 and 47) or by the time post-operative pressures were determined 30 to 75 minutes after recovery from anesthesia (Dogs 43, 44, 48 and 49). In Dog 25, which was not studied during the operation, post-operative observations were made by the indirect method. Twenty-four hours after the fourth moderator nerve was cut, arterial pressure was 151/78[7], while two and a half hours later the pressure had risen to 226/161[7], and there remained. The cardiac rate was about 190 on both occasions.

Thus Dog 25, which probably had normal pressure during the first 24 hours of the post-operative period, developed hypertension between 24 and 26½ hours after operation. In Dog 48 the normal pressure noted at the end of the operation had disappeared in 24 hours, and in Dogs 43 and 49 at the end of 48 hours (Table III).

In Dog 44 the diastolic pressure was definitely elevated in 24 hours, but the systolic pressure did not rise until the fourth post-operative day. In Dog 15 arterial pressure was still normal on the fourth day but had risen strikingly on the sixth post-operative day.

C. *The Development of Chronic Hypertension.* Eight of the ten dogs studied in the unanesthetized state developed hypertension within 48

[7] Average of three determinations.

hours of the operation in which the maximum acute pressor response occurred; all had developed it by the sixth day (Table III). However, in most of the animals arterial pressure continued to mount for several weeks. While two, Dogs 25 and 47, never developed pressures of greater magnitude than those established the day after operation [8], the others attained maximum arterial pressures seven to thirty-one days post-operatively.

TABLE III

Appearance of chronic hypertension after section of four moderator nerves

DOG NO.	ARTERIAL PRESSURE FIRST ROSE ABOVE CONTROL LEVELS	CARDIAC RATE FIRST ROSE ABOVE CONTROL LEVELS	ARTERIAL PRESSURE REACHED MAXIMUM	CARDIAC RATE REACHED MAXIMUM
	days[1]	*days*[1]	*days*[1]	*days*[1]
15	6	1	16	6
25	1	1	1	?[2]
27	1	?[2]	31	?[2]
43	2	1	27	2
44	4/1[3]	1	31	52
46	1[4]	1[4]	14[4]	1[4]
47	1	1	1	2
48	1	1	21	1
49	2	1	7	7
51	1	3	13	24

[1] Days from date of fourth moderator nerve section.
[2] Daily cardiac rates not calculated at this time.
[3] Systolic pressure rose on fourth day, diastolic pressure on first day postoperative.
[4] Days from date of third moderator nerve section.

The tachycardia which followed section of the fourth moderator nerve, was present with much more uniformity on the day following operation than the hypertension; it was observed in every animal except Dog 51, which, in the presence of marked hypertension (348/159) showed an actual bradycardia with cardiac rate of 85 on the first post-operative day. (Control cardiac rate 112, Table IV.) Following section of the first three moderator nerves, very mild transient increases in cardiac rate were sometimes noted on the first post-operative day, especially if the nerve cut had been the left vago-sympathico-depressor

[8] Arterial pressure in Dog 47 was 425+/222 twenty-four hours after operation.

trunk, but these rapidly disappeared, and permanent tachycardia never ensued after these early operations.

A comparison between the maximum systolic and diastolic pressures and cardiac rates attained during the acute pressor response and during the chronic state of hypertension in each dog is made in Table IV. In general, the results are similar, although the average maximum systolic pressure was higher during the chronic stage than during the acute pressor response (337 versus 290 mm. of Hg). The average

TABLE IV

Elevation of arterial pressure and cardiac rate during acute and chronic phases of neurogenic hypertension

DOG NO.	CONTROL UNANESTHETIZED[1]			CONTROL ANESTHETIZED[2]			MAXIMUM IMMEDIATELY AFTER NERVE SECTION			MAXIMUM DURING POST-OPERATIVE PERIOD[3]		
	S.P.	D.P.	C.R.	S.P.	D.P.	C.R.	S.P.	D.P.	C.R.	S.P.	D.P.	C.R.
43	181	85	90	158	92	230	240	142	270	330	182	225
44	204	115	74	195	133	220	352	205	245	372	230	255
46	180	94	116	185	116	225	284	169	247	260	162	225
47	234	127	129	212	138	200	263	155	178	425	222	255
48	131	73	79	154	94	164	247	152	210	270	164	165
49	172	91	114	227	139	180	330	200	255	305	198	210
51	200	102	112	239	143	200	317	181	205	395	213	230
Average.....	186	98	102	196	122	203	290	172	230	337	199	224

S.P. = systolic pressure in mm. Hg; D.P. = diastolic pressure in mm. Hg; C.R. = cardiac rate per minute.

[1] Average of three determinations immediately prior to day of first operation.

[2] Determinations immediately after anesthesia was induced during operation in which the greatest pressor response occurred.

[3] Maximum S.P., D.P. and C.R. not necessarily recorded on the same day.

maximum diastolic pressure was 172 during the acute and 199 during the chronic phase, while the average maximum cardiac rates were 230 and 240, respectively.

D. *Characteristics of Chronic Neurogenic Hypertension.* 1. *General Condition of the Animal.* During the chronic stage of hypertension the dogs appeared entirely normal, save for the Horner's syndrome which appeared following section of the cervical sympathetic fibers on the left and to some extent on the right. The dogs remained active, and there was no evidence of decreased cardiac reserve such as dyspnoea or

easy fatigability, even on strenuous exercise. Likewise, no outward change in temperament suggesting the development of a "hypertensive personality" took place following operation. The apathetic dogs re-

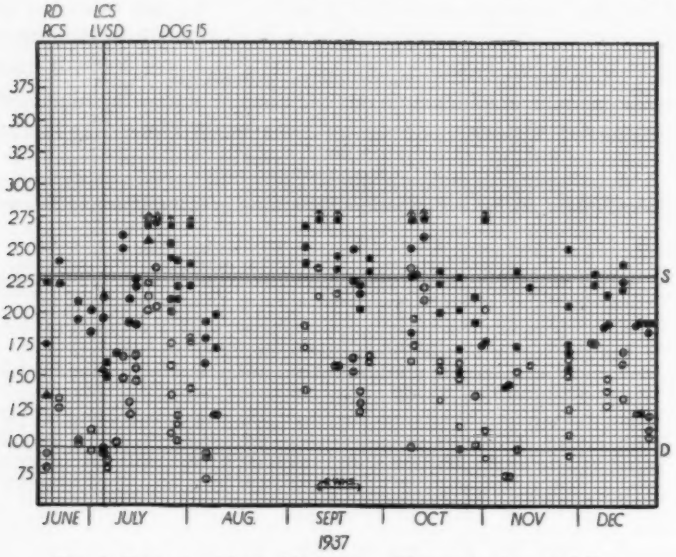


FIG. 3. DOG 15. MALE, MONGREL. WEIGHT 11.5 TO 14.5 KG.

● = systolic pressure, ○ = diastolic pressure, mm. Hg, *indirect* method. ▲ = mean direct arterial pressure. RD = right depressor nerve fibers severed. RCS = right carotid sinus nerve severed. LCS = left carotid sinus nerve severed. LVSD = left vago-sympathico-depressor trunk severed. The systolic and diastolic pressures recorded during the control period were all below the levels S and D. ↑ = the systolic pressure was somewhat above the level indicated on the chart.

After the first two moderator nerves were severed, there was a slight elevation of pressure which tended to subside. Following the third and fourth nerve sections the arterial pressure, especially the diastolic pressure, rose to great heights, and tremendous variability in the pressures obtained appeared. Pressures charted in vertical columns were taken on the same day, at intervals of a few minutes. The first pressure obtained was usually the highest, and the pressure fell progressively during a series of determinations.

mained apathetic, the gay ones gay. High strung dogs did not become more restless or difficult to handle. Appetite and weight were unaffected, and the food intake of the dogs was not different in the

hypertensive than the normal state. There was nothing to suggest gross alteration of the metabolic requirements of the body. General-

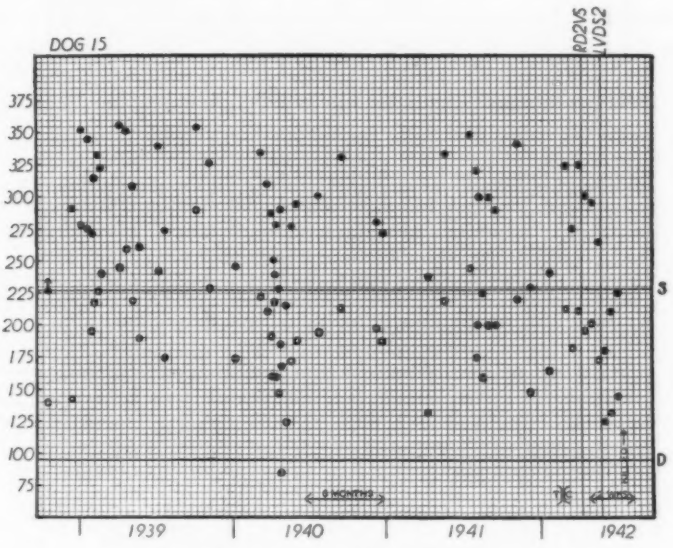


FIG. 4. DOG 15. AS IN FIG. 3

● = systolic pressure, ○ = diastolic pressure, mm. Hg, *direct* method; RD2VS = right vago-sympathico-depressor trunk severed, the depressor fibers for the second time. LVDS2 = left vago-sympathico-depressor trunk severed for the second time. T C indicates a change in the scale of the time axis at the line between the two letters. S and D as in Fig. 3. Marked hypertension and variability of arterial pressure persisted, and were still present four years nine months after it first appeared. Total section of the right vago-sympathico-depressor trunk did not produce any immediate rise in arterial pressure, and when both vago-sympathico-depressor trunks were sectioned, the pressure fell and the dog was killed. (Between Fig. 3 and Fig. 4 nearly a year is omitted. Mean direct arterial pressures during that period were: 1–21–38, 228 mm. Hg; 4–6–38, 190 mm. Hg; 7–1–38, 234—200 mm. Hg).

ized quivering, which is often noted in dogs with renal hypertension after renal artery constriction did not follow moderator nerve section.

2. *Duration of Hypertension.* All of the ten animals in which the four moderator nerves were successfully sectioned developed and main-

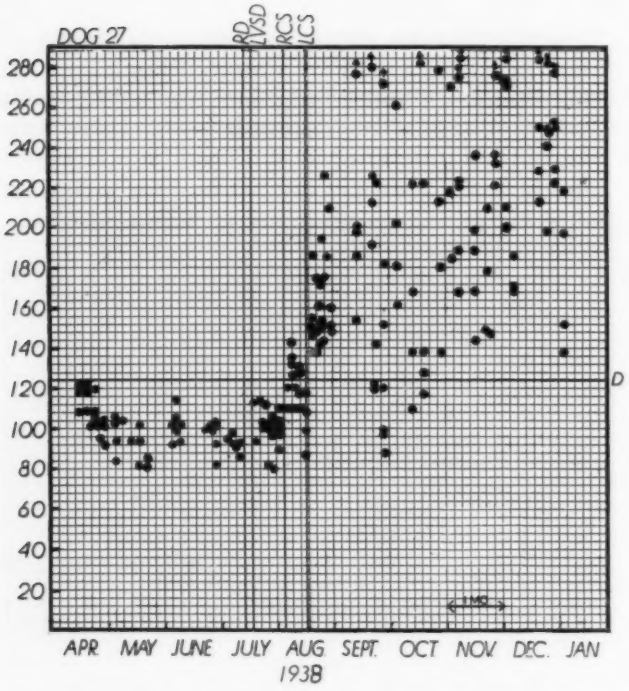


FIG. 5. DOG 27. FEMALE, MONGREL BULL TERRIER TYPE. WEIGHT 10.5–13.2 KG.

● = diastolic pressure, mm. Hg, *indirect* method. RD = right depressor nerve fibers severed. LVSD = left vago-sympathico-depressor trunk severed. RCS = right carotid sinus nerve severed. LCS = left carotid sinus nerve severed. D = the diastolic pressures recorded during the preoperative control period were all below this level. ↑ = the diastolic pressure was somewhat above the level indicated on the chart.

There was a slight increase in the height of the diastolic pressure after the third operation, without any increase in the variability of the pressure. After cutting the fourth moderator nerve, there was a great increase in the height of the diastolic pressure, and at the same time tremendous variability in the pressures obtained appeared. Pressures charted in vertical columns were taken on the same day, usually at intervals of a few minutes. The first pressure obtained was usually the highest, and the pressure fell progressively during a series of determinations. The relation of the diastolic pressure to the cardiac rate in this animal is illustrated in Fig. 19. The systolic pressure ranged between 160 and 192 during the final month of the control period. It did not become elevated following the third operation. Following the fourth operation the systolic pressure became elevated and was frequently too high to be recorded by this method; on this account systolic pressure is not charted.

tained unequivocal hypertension accompanied by tachycardia for an appreciable period of time. Of the three dogs observed over a span of years, Dogs 15 and 27 (Figs. 3, 4, 5 and 6) continued to have hypertension of unabated intensity for periods of 4 years, 9 months and 3 years, 8 months respectively. They were killed while the hypertension was

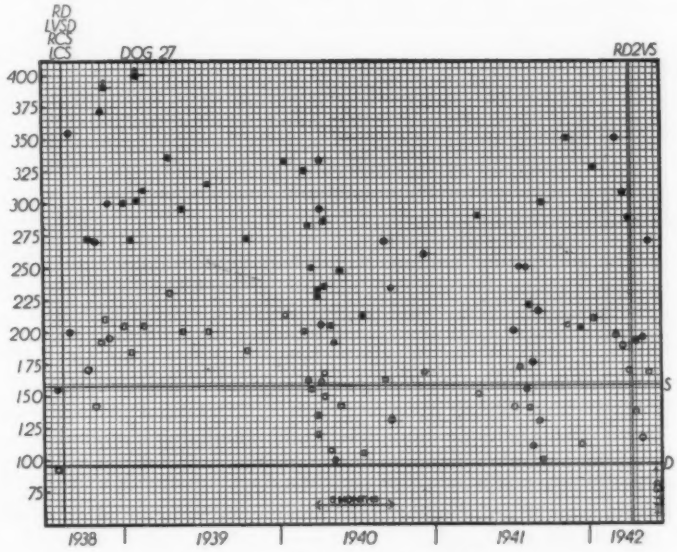


FIG. 6. DOG 27. AS IN FIG. 5

● = systolic pressure, ○ = diastolic pressure, mm. of Hg, *direct* method. RD2VS = right vago-sympathico-depressor trunk severed, the depressor fibers for the second time. Other symbols as in Fig. 5. After the four moderator nerves were severed the systolic and diastolic pressures rose to great heights and remained elevated for three years eight months. No increase in arterial pressure occurred after severing the right depressor fibers for the second time.

still well marked; tachycardia had persisted to the end. Dogs 44, 47, 48 and 49 (Figs. 7, 8, 9, 10 and 11) were killed one to ten months after the development of hypertension which was present up to the time of death. Severe illness interrupted the course of the hypertension in Dog 51 (Fig. 12). In Dogs 25, 43 and 46 (Figs. 13, 14, 15, 16, 17 and 18) the hypertension which initially appeared was not well maintained;

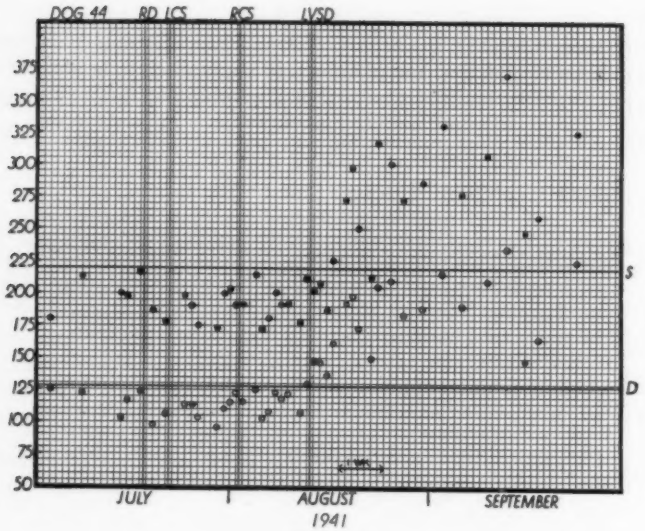

DOG 44
BD ICS
RCS
LVSD
375
350
325
300
275
250
225
200
175
150
125
100
75
50
S
D
JULY
AUGUST
SEPTEMBER
1941

FIG. 7

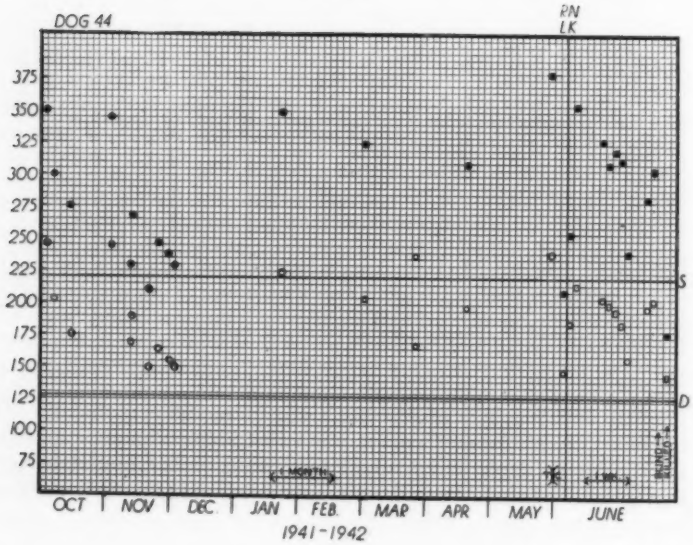

DOG 44
RN
LK
375
350
325
300
275
250
225
200
175
150
125
100
75
S
D
OCT
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUNE
1941-1942

FIG. 8

further section of depressor fibers in the right vago-sympathico-depressor trunk once more increased the hypertension in Dogs 25 and 46, and Dog 43 was found at autopsy to have well marked regeneration of the left vago-sympathico-depressor trunk. There was, therefore, no indication that this form of hypertension is a transitory one, unless nerve regeneration occurs.

3. *Cardiac Rate.* In every dog the chronic stage of neurogenic hypertension was characterized by tachycardia, which at times rose to extreme heights. (Table V) The tachycardia appeared promptly after section of the fourth moderator nerve, even when the onset of hypertension was delayed, and only diminished appreciably in those animals in which the hypertension largely subsided.

4. *Correlation of Arterial Pressure with Cardiac Rate.* One of the striking features of neurogenic hypertension was the close correlation between the arterial pressure and the cardiac rate. This was expecially well illustrated in the two dogs, 15 and 27, which showed persistent, variable, but usually well marked hypertension over the course of several years. In Figure 19, eighty-two diastolic pressures determined by the indirect method on Dog 27 are plotted against simultaneously recorded cardiac rates.[9] A high degree of correlation is demonstrated; very low diastolic pressures only occurred when the cardiac rate was

[9] Systolic pressures were not plotted because they were often so high that they were inaccurate or unobtainable. The upper limit of accurate measurement by this method lies between 250 and 270 mm. The highest diastolic pressures were probably somewhat distorted.

FIGS. 7 (UPPER) & 8 (LOWER). DOG 44. FEMALE, HOUND. WEIGHT 12.7 KG. ● = systolic pressure, ○ = diastolic pressure, mm. Hg, *direct* method. RD = right depressor fibers severed. LCS = left carotid sinus nerve severed. RCS = right carotid sinus nerve severed. LVSD = left vago-sympathico-depressor trunk severed. RN = right nephrectomy. LK = left renal artery constricted. T C indicates a change in the scale of the time axis at the line between the two letters. The systolic and diastolic pressures recorded during the pre-operative control period were all below the levels S and D. Following the fourth operation there was a gradual elevation of systolic and diastolic pressures to a high level. The hypertension was well maintained for over nine months. After removal of one kidney and constriction of the other renal artery there was no definite change in the blood pressure. The dog suddenly developed the syndrome of malignant hypertension two weeks after the final operation and was killed.

low, high ones when the cardiac rate was high. The same linear relationship is seen in Figure 20; here direct measurements were made on

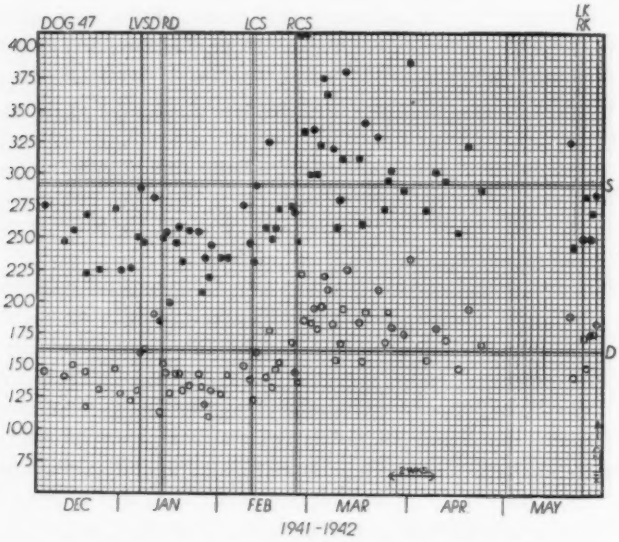


FIG. 9. DOG 47. FEMALE, MONGREL, WEIGHT 13 KGS.

● = systolic pressure, ○ = diastolic pressure, mm. Hg, *direct* method. LVSD = left vago-sympathico-depressor trunk severed. RD = right depressor fibers severed. LCS = left carotid sinus nerve severed. RCS = right carotid sinus nerve severed. LK = left renal artery constricted. RK = right renal artery constricted. The systolic and diastolic pressures recorded during the preoperative control period were all below the levels S and D. Following the first and third operations the diastolic pressure was occasionally higher than the control level, and a single high systolic reading is recorded. Immediately after the fourth moderator nerve was cut well-marked hypertension appeared together with increased variability in the daily pressures. Moderate increase in pressure levels was present at the end of three months. Following constriction of both renal arteries pressure levels did not rise further at a time when there was increasing nitrogen retention.

forty-two occasions on Dog 15, and there is no distortion of the upper levels of pressure by this method. In contrast, the diastolic pressures were plotted against simultaneous cardiac rates in a dog with chronic

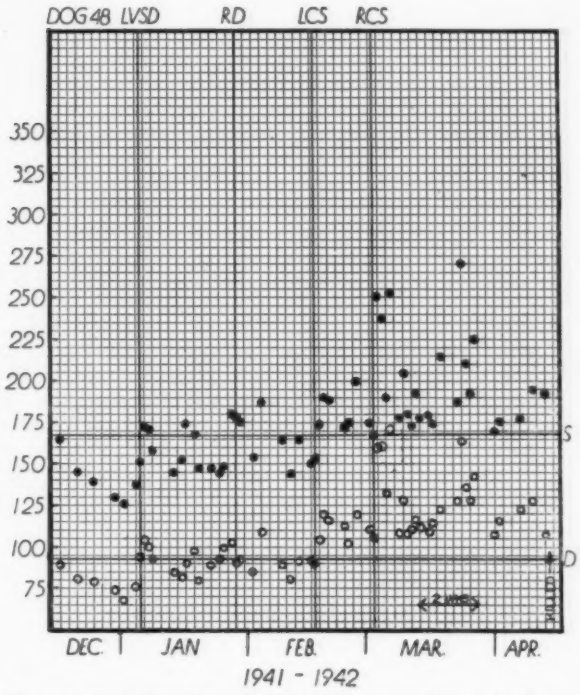


FIG. 10. DOG 48. FEMALE, SMALL SPITZ. WEIGHT 10 KGS.

● = systolic pressure, ○ = diastolic pressure, mm. Hg, *direct* method. LVDS = left vago-sympathico-depressor trunk severed. RD = right depressor fibers severed. LCS = left carotid sinus nerve severed. RCS = right carotid sinus nerve severed. The systolic and diastolic pressures recorded during the pre-operative control period were all below the levels S and D. Following the first operation slight elevation of arterial pressure appeared and persisted, and following the third operation the hypertension became more definitely sustained. Immediately after severing the fourth moderator nerve there was a marked heightening of the hypertension and increased variability in pressure which persisted for a month, after which pressure levels, while still somewhat elevated, became more stable.

renal hypertension from bilateral constriction of the renal arteries which was studied for a similar period of time (Fig. 21, Dog 5). It will be seen that both pressures and cardiac rates were much more stable than

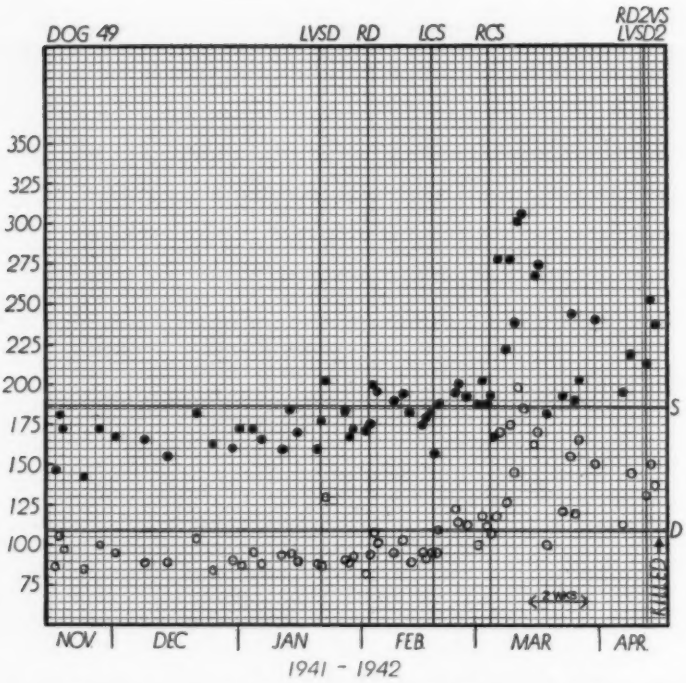


FIG. 11. DOG 49. FEMALE, MONGREL. WEIGHT, 15.5 KG.

● = systolic pressure, ○ = diastolic pressure, mm. Hg, *direct* method. LVSD = left vago-sympathico-depressor trunk severed. LVDS2 = the same, for the second time. LCS = left carotid sinus nerve severed. RCS = right carotid sinus nerve severed. RD2VS = entire right vago-sympathico-depressor trunk severed, the right depressor fibers for the second time. The systolic and diastolic pressures recorded during the pre-operative control period were all below the levels S and D. Following the first operation the blood pressure was elevated on a single occasion. Following the second operation the systolic, and, following the third operation, the diastolic pressures rose a little but remained stable. After the fourth operation extreme hypertension appeared, which moderated but was still well marked after two weeks. Subsequent bilateral section of both vago-sympathico-depressor trunks had little immediate effect on the blood pressure.

in neurogenic hypertension and that the height of one was not positively correlated with the height of the other. Thus in Dog 5 the ***highest*** diastolic pressure was associated with a slightly ***slower*** cardiac

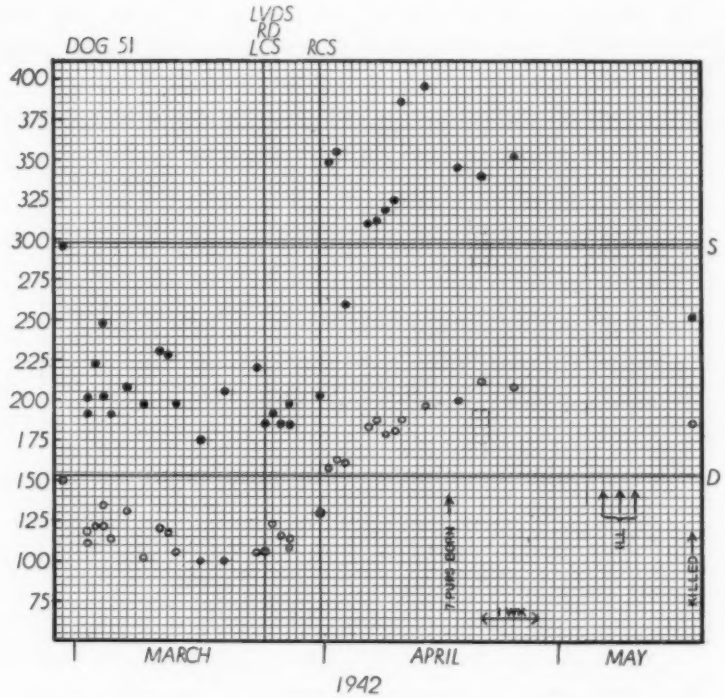


FIG. 12. DOG 51. FEMALE, GERMAN SHEPHERD. WEIGHT 16 KG.

● = systolic pressure, ○ = diastolic pressure, mm. Hg., direct method. LVDS = left vago-sympathico-depressor truck severed. RD = right depressor fibers severed. LCS = left carotid sinus nerve severed. RCS = right carotid sinus nerve severed. The systolic and diastolic pressures recorded during the preoperative control period were all below the levels S and D. (Actually all the control readings except the first, when the dog was untrained, were considerably below these levels.) The first three moderator nerves were cut at a single operation; blood pressure was not significantly altered. Immediately after the fourth operation well marked hypertension appeared, which was unaffected by the animal giving birth to seven puppies. Later the animal became extremely ill, with weakness and emaciation, and was finally killed. At autopsy heart worm and tapeworm were found.

rate than was the *lowest* diastolic pressure. Conversely, the highest and lowest cardiac rates (205, 120) were associated with rather similar diastolic pressures (133, 120 mm. Hg).

The intimate relationship of arterial pressure to cardiac rate was brought out in many other ways during these studies. Following

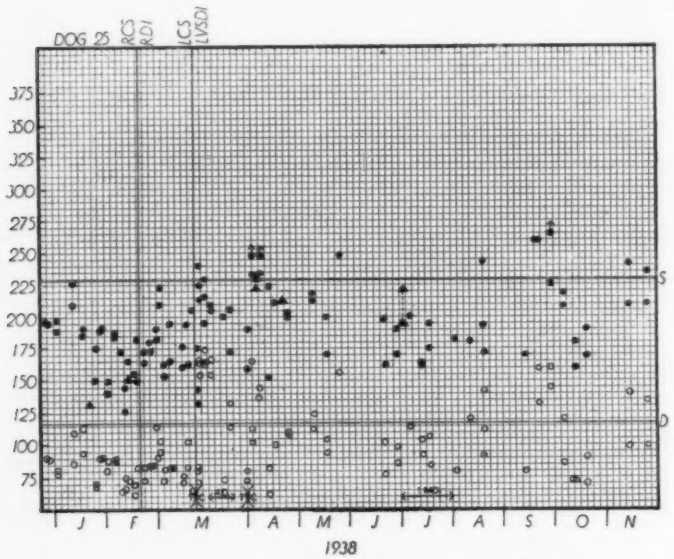


FIG. 13. DOG 25. FEMALE, MONGREL. WEIGHT 12 KG.

● = systolic pressure, ○ = diastolic pressure, mm. of Hg, *indirect* method. ▲ = mean *direct* pressure, mm. of Hg. Two such triangles connected by dotted line indicate the extremes of pressure on a given day. RCS = right carotid sinus nerve severed. RD1 = right depressor fibers severed the first time. LCS = left carotid sinus nerve severed. LVSD1 = left vago-sympathico-depressor trunk severed the first time. T C indicates a change in the scale of the time axis at the line between the two letters. The systolic and diastolic pressures recorded during the pre-operative control period were all below the levels S and D (on all but two occasions, considerably below.) The letters on the time axis indicate months. After the four moderator nerves were cut in two stages, there was a marked rise in diastolic and mean arterial pressure, a less striking rise in systolic pressure. There was great variability in pressure levels; very high pressures were noted only occasionally.

section of the fourth moderator nerve, arterial pressure and cardiac rate increased simultaneously to produce the acute pressor response (Fig. 2). In dogs showing exceeding lability of arterial pressure, the pressure and

cardiac rate usually varied in the same direction, as in Figure 22, where the pressure and cardiac rate were shown to decrease synchronously over a period of nearly 2 minutes from the moment of femoral puncture. Likewise, in periods when the chronic hypertension had largely sub-

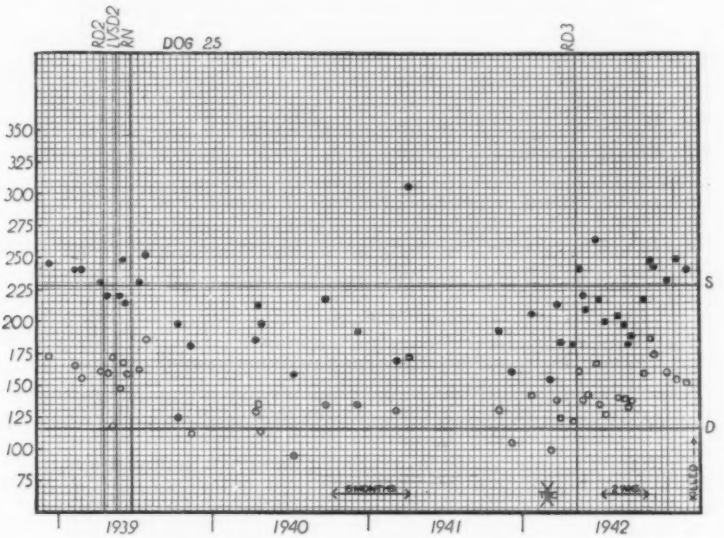


FIG. 14. DOG 25. AS IN FIG. 13

● = systolic pressure, ○ = diastolic pressure, mm. of Hg, *direct* method. RD2 and 3 = right depressor fibers severed the second and third time. LVSD2 = left vago-sympathico-depressor trunk severed the second time. RN = right nephrectomy. T C, S and D as in Fig. 13. Moderate hypertension, chiefly diastolic, persisted for slightly over four years. Slight increase in pressure levels occurred after severing the right depressor fibers for the third time.

sided, cardiac rate was no longer excessive (Table V, Dogs 25, 43, 46, 48 and 49).

From these findings, a rapid cardiac rate appears to be a primary factor in the production of neurogenic hypertension, and the variability of the blood pressure levels seems to depend upon variability of cardiac rate, in contrast to renal hypertension. That other mechanisms are also active in the production of variation in the elevated pressure levels

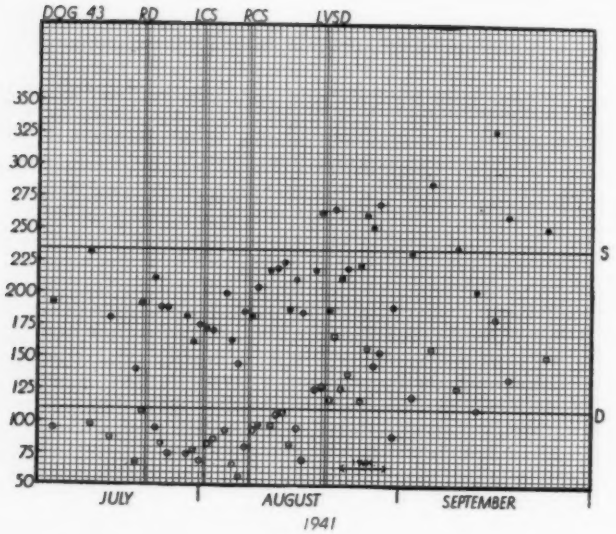

DOG 43
RD
LCS
RCS
LVSD
350
325
300
275
250
225
200
175
150
125
100
75
50
S
D
JULY
AUGUST
SEPTEMBER
1941

FIG. 15

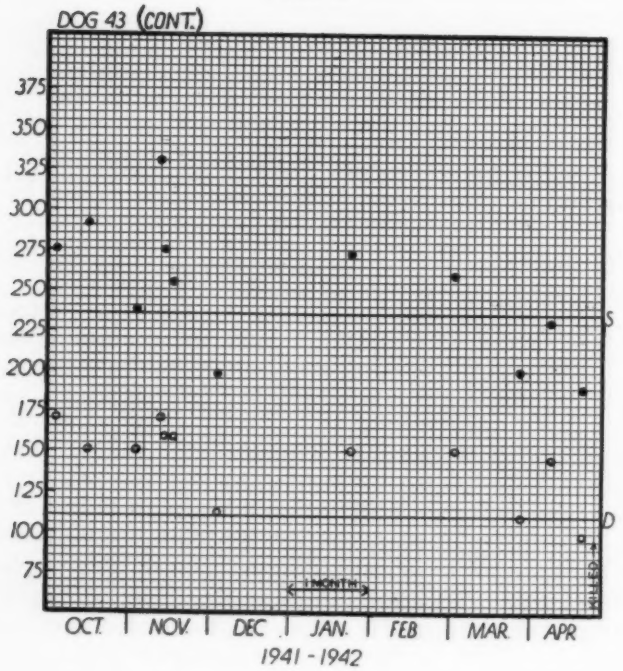

DOG 43 (CONT.)
375
350
325
300
275
250
225
200
175
150
125
100
75
S
D
1 MONTH
OCT.
NOV.
DEC
JAN.
FEB
MAR.
APR
1941 - 1942

FIG. 16

is shown by occasional instances in which the arterial pressure rose while the cardiac rate was falling (Fig. 23). Such examples were far less frequent than the positive correlation of arterial pressure and cardiac rate shown in Figure 22, but they illustrate the fact that other factors, such as changing degrees of peripheral vaso-constriction, are capable of playing their part in neurogenic hypertension. The degree of participation of these secondary factors in blood pressure regulation under such circumstances seems to vary from animal to animal. Thus in Dogs 15 and 27, there was such a high degree of correlation between cardiac rate and arterial pressure that the remainder of the factors concerned with the maintenance of blood pressure may be inferred to have been exceedingly stable. In Dog 47, on the other hand, while the general positive correlation between cardiac rate and arterial pressure did exist (Fig. 24), there seemed to be considerably more latitude and variability in this correlation than in other animals. Inspection of examples of the daily records taken during the first five weeks of neurogenic hypertension in Dog 47 (Fig. 25) show marked differences in the systolic, diastolic and pulse pressures and cardiac rates, with Traube Hering waves, sinus arrhythmia and pulsus alternans being present on some occasions and not on others, indicating quite wide variations in the hemodynamic relationships on different occasions. Since Dog 47 had the highest normal arterial pressures (Fig. 9) it may

FIG. 15 (UPPER) & 16 (LOWER). DOG 43. FEMALE, WIREHAIRED TERRIER. WEIGHT 12.7 KG.

● = systolic pressure, ○ = diastolic pressure, mm. Hg, *direct* method. RD = right depressor fibers severed. LCS = left carotid sinus nerve severed. RCS = right carotid sinus nerve severed. LVSD = left vago-sympathico-depressor trunk severed. The systolic and diastolic pressures recorded during the preoperative control period were all below the levels S and D. Following the third operation instability of blood pressure appeared (not charted). Pressure variations of 10 to 25 mm. were observed during the recording of arterial pressure for 1 to 3 minutes which had not been present previously. The blood pressure was elevated for the first time immediately before the anesthetic for the fourth operation was administered; the dog undoubtedly recognized that operative preparations were afoot, and was apprehensive. Following the fourth operation a definite hypertension of moderate degree developed which persisted a little over six months and then disappeared. At autopsy no break in the continuity of the left vago-sympathico-depressor trunk was found, although it had once been transected.

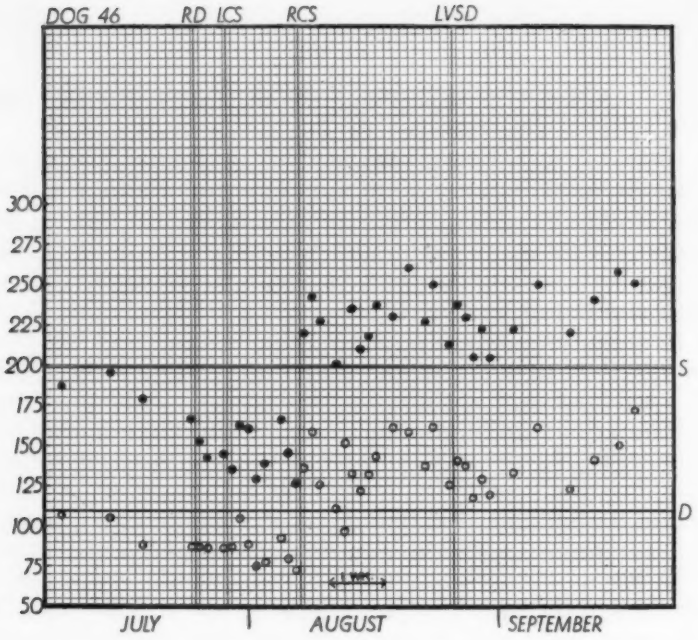

DOG 46
RD
LCS
RCS
LVSD
300
275
250
225
200
175
150
125
100
75
50
S
D
1 WK.
JULY
AUGUST
SEPTEMBER
1941

FIG. 17

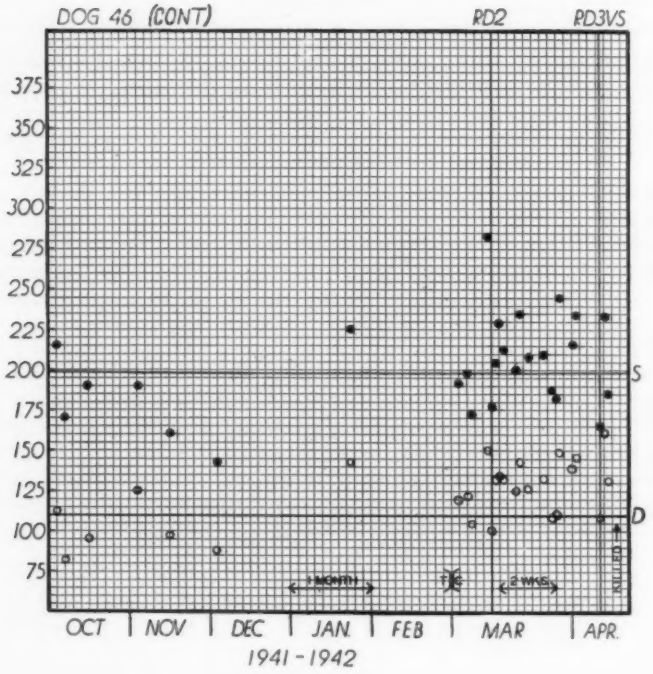

DOG 46 (CONT)
RD2
RD3VS
375
350
325
300
275
250
225
200
175
150
125
100
75
S
D
1 MONTH
2 WKS.
OCT
NOV
DEC
JAN.
FEB
MAR
APR.
1941 - 1942

FIG. 18

well be that this animal had a more reactive vasoconstrictor mechanism than the others.

5. *Relationship of Pressure Levels During the Normal State to the Height of the Subsequent Hypertension.* It will be noted in Table IV that the control pressures varied considerably from dog to dog. For example, Dog 47, tense, immobile and undemonstrative, showed consistently high levels during the normal state; the last three averaged 234/127 (Fig. 9). This was in striking contrast to the low pressures observed in Dog 48, a friendly, relaxed, playful animal, with an average pressure of 131/73 on the three days before the first operation (Fig. 10). These two dogs had control cardiac rates of 129 and 79, respectively. The question arose whether dogs characterized by high arterial pressures during the normal state would tend to be more or less reactive to nerve section than dogs with normally low pressures. In other words, would the animals with normally high levels show responses of greater magnitude to nerve section, suggesting that they were hyperreactors to pressor stimuli, or would the fact that their pressures were already elevated, possibly from neurogenic causes, leave decreased latitude for a subsequent rise? In order to determine the answer to this question, the dogs were divided into high pressure and low pressure groups (Table VI). Inspection of Table VI reveals no clear cut evidence that the

FIGS. 17 (UPPER) & 18 (LOWER). DOG 46. FEMALE, MONGREL. WEIGHT 9 KG.

● = systolic pressure, ○ = diastolic pressure, mm. Hg, *direct* method. RD = right depressor fibers severed. LCS = left carotid sinus nerve severed. RCS = right carotid sinus nerve severed. LVSD = left vago-sympathico-depressor trunk severed. RD2 and RD3VS = right depressor fibers severed for a second and third time, on the last occasion, the entire right vago-sympathico-depressor trunk being severed. T C indicates a change in the scale of the time axis at the line between the two letters. The systolic and diastolic pressures recorded during the pre-operative control period were all below the levels S and D. Following the third operation there was an abrupt elevation of systolic and diastolic pressures which was not increased after the fourth operation. The hypertension persisted almost two months, then subsided. Increased instability of pressure was still present, however, and occasional high readings appeared. Following a second sectioning of the right depressor fibers, moderate hypertension again appeared. Section of the entire right vago-sympathico-depressor trunk produced no further rise in pressure.

TABLE V

Effect of moderator nerve section on cardiac rate

DOG NO.	RANGE OF CARDIAC RATE PER MINUTE				
	Control Period	Following Section of Four Moderator Nerves			
		Before hypertension appeared	During established hypertension	After hypertension had largely subsided	Remarks
15	104–120	136–160	120–250		Following terminal section of right V-S-D. trunk, C.R. range was 180–203. After left trunk was also cut, C.R. range was 135–152.
25	105–128	187–204	150–244	150–180	None of the later redivisions of the depressor nerves affected the C.R. appreciably.
27	97–105		110–240		Following terminal section of right V-S-D. trunk, C.R. range was 120–135.
43	70–132	185	85–225	98–118	
44	62–87	158–185	110–255		Following removal of R.K. and constriction of left renal artery, C.R. range during benign phase was 112–232, during benign phase was 112–232, during malignant phase 175.
46	72–140		120–225	105–160	Following second transection of R.D. fibers, C.R. range was 117–170. After terminal section of right V-S-D. trunk, C.R. range was 180–195.
47	110–155		120–270		Following constriction of both renal arteries C.R. range was 85–150 (malignant phase).
48	75–118		98–165	90–130	
49	82–145	153	153–210	120–150	Following terminal transection of both V-S-D. trunks, C.R. range was 144–156.
51	100–160		85–230		Following fourth operation, C.R. was 85, 102 and 169 on the first three days, although arterial hypertension was marked. From then until parturition, C.R. range was 207–215. Thereafter, it was 190–230. Terminal C.R. was 203.

C.R. = cardiac rate; V-S-D. = vago-sympathico-depressor; R.D. = right depressor; R.K. = right kidney.

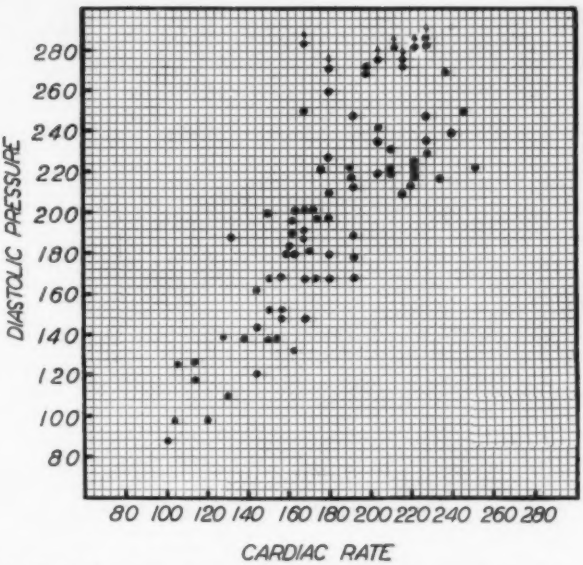


FIG. 19. DOG 27. CORRELATION OF DIASTOLIC PRESSURE WITH CARDIAC RATE IN CHRONIC NEUROGENIC HYPERTENSION

Eighty-two observations made between August 1938 and October 1939, of the diastolic pressure and cardiac rate determined indirectly by the method of Kolls and Cash.

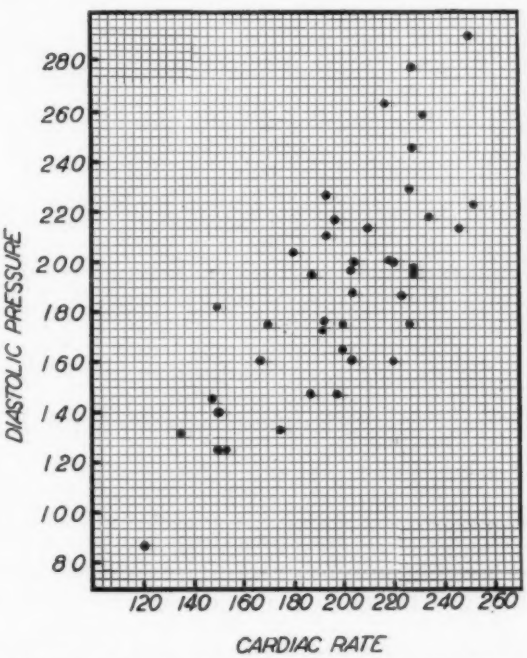


FIG. 20. DOG 15. CORRELATION OF DIASTOLIC PRESSURE WITH CARDIAC RATE IN CHRONIC NEUROGENIC HYPERTENSION

Forty-two observations made between October 1938 and April 1942 of the initial diastolic pressure and cardiac rate immediately following arterial puncture by the direct Hamilton method.

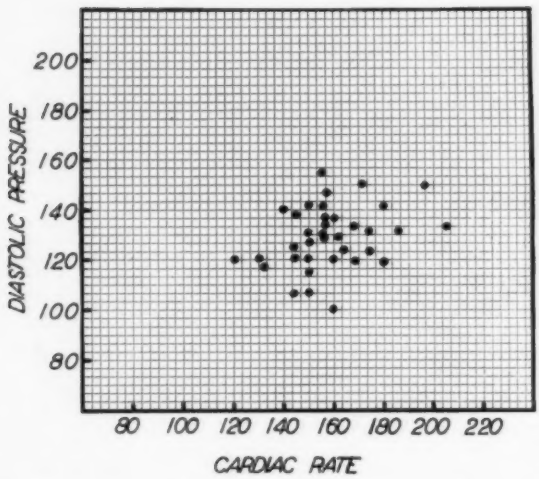


FIG. 21. DOG 5. CORRELATION OF DIASTOLIC PRESSURE WITH CARDIAC RATE IN CHRONIC RENAL HYPERTENSION

Thirty-six observations made between January 1939 and January 1942 of the initial diastolic pressure and cardiac rate immediately following arterial puncture by the direct Hamilton method.

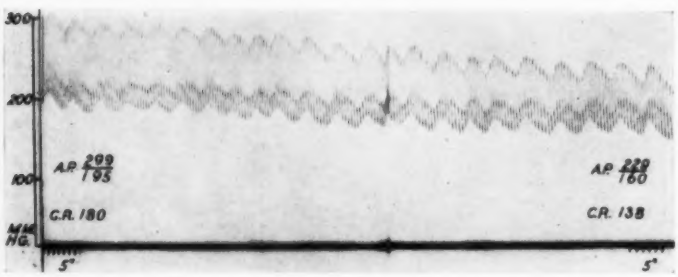


FIG. 22. DOG. 27

Instability of arterial pressure and cardiac rate in chronic neurogenic hypertension. Both declined simultaneously in this record during the first two minutes after femoral artery puncture. A.P. = arterial pressure; C.R. = cardiac rate.

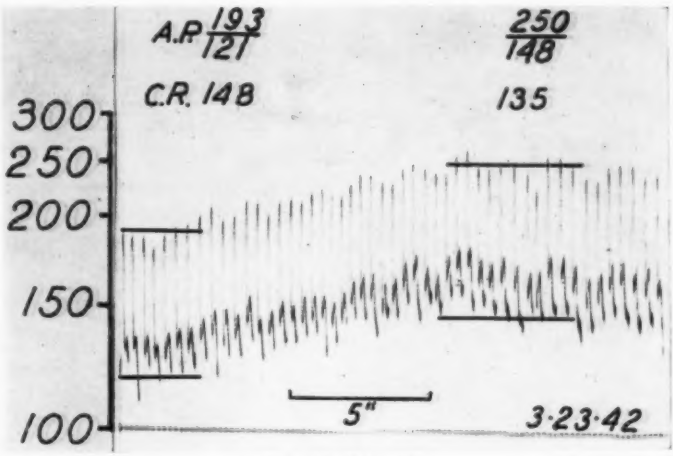


FIG. 23. DOG 49

The arterial pressure rose sharply in this instance while the cardiac rate decreased.

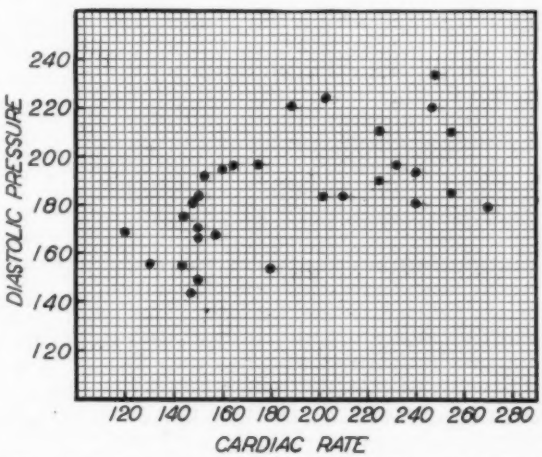


FIG. 24. DOG 47. CORRELATION OF DIASTOLIC PRESSURE WITH CARDIAC RATE IN CHRONIC NEUROGENIC HYPERTENSION

Thirty observations made between February and May 1942, of the initial diastolic pressure and cardiac rate immediately following arterial puncture by the direct Hamilton method.

degree of response to moderator nerve section is in any way influenced by the height of the arterial pressure levels when the dog is in the

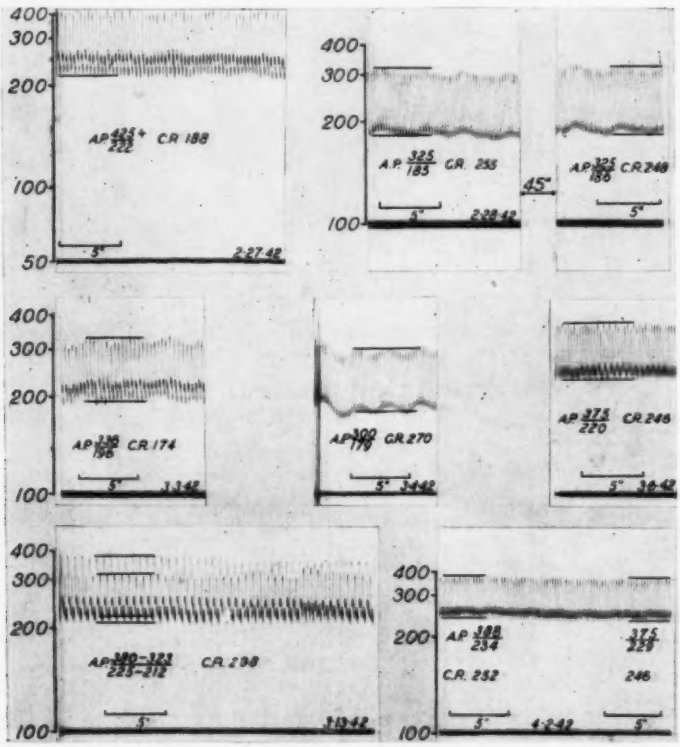


FIG. 25. DOG 47

Examples of the daily records of arterial pressure and cardiac rate taken during the five weeks following the section of the fourth moderator nerve on February 26, 1942.

normal state. It is of interest that the average control cardiac rates were practically the same, 105 and 100, in Groups I and II.

E. *The Effect of Constriction of Renal Arteries upon Chronic Neurogenic Hypertension.* Renal artery constriction was carried out in two dogs with well established neurogenic hypertension.

Dog 44 (Figs. 7 and 8). Well marked but variable hypertension and tachycardia, ranging up to 380/239 and 258, respectively, were present during the nine months following moderator nerve section. On June 2, 1942, the right kidney was removed, and a Goldblatt clamp was placed on the left renal artery, partially occluding it. The non-protein nitrogen of the blood was 56 mgm. per cent on June 3 and 26 mgm. per cent on June 4. The dog was active and seemed entirely normal for the next two weeks. Arterial pressures and cardiac rates remained high and labile, and no change of any sort attributable to the clamping could be detected. On June 17 the dog had become suddenly blind and was definitely ill. Vomiting, diarrhoea and prostration appeared, and on June 18 the dog was killed, showing the classical picture of malignant hypertension described by Goldblatt (50). There was a terminal drop in arterial pressure to 178/144 associated with the moribund condition of the animal, but the cardiac rate remained around 175.

TABLE VI

Comparison of reactivity to moderator nerve section of dogs with high and low arterial pressures in the normal state[1]

GROUP	DOG NOS.	NORMAL STATE			MAXIMUM RISE DURING CHRONIC HYPERTENSION					
		S.P.	D.P.	C.R.	S.P.		D.P.		C.R.	
		mm.	mm.	/min.	mm.	%	mm.	%	/min.	%
I. High Normal Arterial Pressures	44, 47, 51	213	115	105	+185	+82	+107	+92	+142	+135
II. Low Normal Arterial Pressures	43, 46, 48, 49	166	86	100	+123	+74	+94	+109	+107	+107

[1] Only the average values are included for the sake of brevity; inspection of the individual values for each dog adds nothing of importance.
S.P. = systolic pressure; D.P. = diastolic pressure; C.R. = cardiac rate.

Dog 47 (Fig. 9). During the three months following moderator nerve section, arterial pressure ranged up to 425+/222 and the cardiac rate up to 255; at times the pressure fell into the upper level of normal range, and tachycardia was not very conspicuous. On May 25, 1942 both renal arteries were partially occluded by Goldblatt clamps. On May 26 the dog was quivering and voiding large quantities of urine. The non-protein nitrogen of the blood was 46 mgm. per cent, and two days later had risen to 144 mgm. per cent. Since the dog was vomiting and obviously in uremia, it was killed on May 29, four days after the operation. The arterial pressure remained unchanged during this period, but the cardiac rate was 85, 150, 105 and 123 on the four post-operative days. Cardiac rates as low as 85 and 105 had not been observed among the thirty-one recordings of cardiac rate during the three months of pure neurogenic hypertension (Table V).

While these experiments are only suggestive, it is worth noting that neither dog showed any increase in the elevation of arterial pressure,

despite the fact that both dogs developed the clinical picture of experimental malignant hypertension, which is usually accompanied by a well-marked rise in pressure. One dog exhibited definite slowing of the cardiac rate during the malignant stage. The significance of these findings in terms of vasoconstriction and circulating angiotonin awaits further investigation.

F. *The Effect of Pregnancy upon Neurogenic Hypertension.*

Dog 51 (Fig. 12). A healthy looking animal of Belgian shepherd type, was the last dog added to the colony, and the only one in which the quarantine period was omitted. As a result, the operations were carried out while the dog was unsuspectedly pregnant. Well marked hypertension appeared the day after the fourth moderator nerve was sectioned. On the sixteenth post-operative day, seven puppies were born; three days before this event arterial pressure was 395/197 and the cardiac rate was 207, while the day following delivery the pressure was 345/200 and the cardiac rate 190. A week later they were 353/209 and 230, respectively. During the lactation period the dog became extremely emaciated and weak, so that it finally was killed. On the day of death arterial pressure was 250/185, cardiac rate 203. At autopsy heavy infestation with tapeworm and heart worm was present.

Pregnancy, therefore, does not interfere with the development of neurogenic hypertension, nor is the latter state incompatible with giving birth to viable young. There was no striking difference in arterial pressure, cardiac rate or pulse pressure before and after parturition.

DISCUSSION

These studies reveal that dogs with neurogenic hypertension present a characteristic picture which is totally unlike that seen in renal hypertension. The acute and chronic phases of neurogenic hypertension are very similar in the degree of blood pressure elevation attained, in the tachycardia which accompanies it, and in the variability of both pressure levels and cardiac rate. It therefore seems that Heymans was entirely correct in assuming that the two stages of neurogenic hypertension derive their characteristics from the same fundamental physiological mechanisms.

On the other hand, it is also true that a latent period of normal arterial pressure lasting one or more days usually exists between the appearance of the acute pressor response and the onset of the chronic

stage of hypertension, and that it may be a matter of weeks before the hypertension again reaches its maximum intensity. The fact that a well-marked decline in arterial pressure is first noted within a few minutes of the peak of the pressor response, while the animal is still in excellent condition under anesthesia, makes it very unlikely that the latent period represents a non-specific post-operative asthenic state, and points to some definite depressor influence implicit in the hemodynamic relationships themselves as the cause of this interruption in the hypertensive state.

While this depressor influence has not yet been experimentally identified, the following hypothesis as to its origin may be offered. It has been seen that the hypertension is closely associated with the cardiac rate, and varies directly with it. The inference is that the hypertension depends upon an increased cardiac output per minute, and that the cardiac output per beat remains relatively constant, while the cardiac rate, cardiac output per minute and arterial pressure vary in unison in one direction or the other. (Other factors, such as peripheral vasomotor tone, must also be assumed to stable, at least in algebraic summation, whenever arterial pressure is varying directly with cardiac rate.) It seems reasonable to suppose, however, that the abrupt onset of tachycardia with rate ranging from 200 to 270 beats per minute during the acute response, throws a sudden severe burden upon the functional capacity of the heart, so that the cardiac output per beat begins to diminish within a few minutes of the peak of the acute pressor response. Even though the tachycardia persists, the cardiac output per minute would fall, and the hypertension tend to disappear. The latent period of normal pressure intervening between the acute and chronic stages would then represent a period during which the normal heart becomes readjusted to the altered hemodynamics of the organism and the cardiac output per beat gradually increases. The length of time necessary to accomplish this would vary with the constitution of the animal, and while moderate hypertension might reappear within a day or two of the final operation, several weeks might elapse before the return of full cardiac efficiency. It would appear, then, that the cardiac output in this form of hypertension is probably augmented, in contrast to the cardiac output in renal hypertension which has been shown to be unaltered (42).

Doubt has been expressed in many quarters as to whether neurogenic hypertension is "true" hypertension because of the tendency of the arterial pressure to fall toward normal as the animal rests on the table. There seems to be no question but that the arterial pressure in a given animal with neurogenic hypertension may be normal or near normal during a considerable proportion of the time, in contradistinction to dogs with the fixed type of hypertension induced by renal artery constriction. Yet the important fact remains that at any time during the daily life of the dog when the animal is not asleep or under absolutely basal conditions, the arterial pressure rises paroxysmally in an entirely abnormal manner. This situation is not unlike that seen in many cases of human hypertension; Ayman and Goldshine (43) have been particularly emphatic in pointing out the role of visits to the physician's office in producing "artificially" high blood pressure levels. It must be stressed that these animals are not merely nervous or excited; they are thoroughly trained and accustomed to the procedures involved in blood pressure measurement, so that they lie quietly on the table without any struggling or resistance. Their training is attested to by the consistent blood pressure levels recorded before the operations. Yet in these same animals, after removal of the moderator mechanism, it becomes apparent that the sympathetic medullary centers have been constantly subjected to a barrage of impulses which were successfully inhibited when the animal was in its normal state. This type of hypertension permits the study of the significance of these impulses, which are insufficient to disturb the general composure of the animal, yet the presence of which is accurately reflected in the height of the arterial pressure and cardiac rate.

CONCLUSIONS

1. The acute and chronic pressor responses are of similar magnitude and general character in a given animal.

2. A depressor influence usually appears within a few minutes of the maximal acute pressor response following moderator nerve section, which brings the pressure down to normal for a day or two and which may be present in some degree for several weeks.

3. Chronic hypertension usually appears within 48 hours after complete moderator nerve section, and disappears only if nerve regeneration occurs.

4. Chronic neurogenic hypertension is readily produced, and is characterized by great variability of arterial pressure and cardiac rate, which may persist for years.

5. There is close correlation between the heights of the arterial pressure and the cardiac rate, which suggests that increased cardiac output is an important factor in the hemodynamics of neurogenic hypertension. This correlation is not present in renal hypertension, in which cardiac output has been shown to be unaltered.

6. There is no evidence that any humoral mechanism plays an important role in chronic neurogenic hypertension; like acute hypertension, it is essentially reflex in character.

BIBLIOGRAPHY

1. Ayman, D.: The Personality Type of Patients with Arteriolar Essential Hypertension. Am. J. Med. Sci., **186:** 213, 1933.
2. Rennie, T. A. C.: The Role of Personality in Certain Hypertensive States. New Eng. J. Med., **221:** 448, 1939.
3. Menninger, K. A.: Emotional Factors in Hypertension. Bull. N. Y. Acad. Med., **14:** 198, 1938.
4. Saul, L. J.: Hostility in Cases of Essential Hypertension. Psychosomatic Med. **1:** 153, 1939.
5. Hines, E. A., Jr., and Brown, G. E.: The Cold Pressor Test for Measuring the Reactibility of the Blood Pressure: Data Concerning 571 Normal and Hypertensive Subjects. Am. Heart J., **11:** 1, 1936.
6. Hines, E. A., Jr.: The Hereditary Factor in Essential Hypertension. Ann. Int. Med., **11:** 593, 1937.
7. Allen, E. V., Lundy, J. S., and Adson, A. W.: Preoperative Prediction of Effects on Blood Pressure of Neurosurgical Treatment of Hypertension. Proc. Staff Meet., Mayo Clin., **11:** 401, 1936.
8. Allen, E. V., and Adson, A. W.: The Treatment of Hypertension; Medical versus Surgical. Ann. Int. Med., **14:** 288, 1940.
9. Gregory, R., Lindley, E. L., and Levine, H.: Studies on Hypertension: II. The Effect of Spinal Anesthesia on the Blood Pressure of Hypertensive Patients. Its Possible Bearing on the Pathogenesis of Essential Hypertension. Texas Rep. Biol. and Med., **1:** 167, 1943.
10. Alexander, F.: Emotional Factors in Essential Hypertension. Presentation of a Tentative Hypothesis. Psychosomatic Med., **1:** 173, 1939.
11. Cannon, W. B.: Bodily Changes in Pain, Hunger, Fear and Rage. Appleton-Century, New York, 1934.
12. Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W.: Studies on Experimental Hypertension. I. The Production of Persistent

Elevation of Systolic Blood Pressure by means of Renal Ischemia. J. Exp. Med., **59:** 347, 1934.

13. Page, I. H.: The Relationship of the Extrinsic Renal Nerves to the Origin of Experimental Hypertension. Am. J. Physiol., **112:** 166, 1935.
14. Collins, D. A.: Hypertension from Constriction of the Arteries of Denervated Kidneys. Am. J. Physiol., **116:** 616, 1936.
15. Goldblatt, H., Gross, J., and Hanzal, R. F.: Studies on Experimental Hypertension. II. The Effect of Resection of Splanchnic Nerves on Experimental Renal Hypertension. J. Exp. Med., **65:** 233, 1937.
16. Freeman, N. E., and Page, I. H.: Hypertension Produced by Constriction of the Renal Artery in Sympathectomized Dogs. Am. Heart J., **14:** 405, 1937.
17. Page, I. H., and Helmer, O. M.: A Crystalline Pressor Substance (Angiotonin) Resulting from the Reaction between Renin and Renin-Activator. J. Exp. Med., **71:** 29, 1940.
18. Corcoran, A. C., and Page, I. H.: Arterial Hypertension. Correlation of Clinical and Experimental Observations. J. A. M. A., **116:** 690, 1941.
19. Katz, L. N., and Leiter, L.: The Present Conception of "Essential" Hypertension. A Physiological and Clinical Correlation. Psychosomatic Med., **1:** 101, 1939.
20. Hering, H. E.: Die Karotissinusreflexe auf Herz and Gefässe vom normalphysiologischen, pathologisch-physiologischen und klinischen standpunkt. Th. Steinkopff, Dresden, 1927.
21. Heymans, C., Bouckaert, J. J., and Regniers, P.: Le sinus carotidien et la zone homologue cardio-aortique. Doin, Paris, 1933.
22. Bronk, D. W., and Stella, G.: Afferent Impulses in the Carotid Sinus Nerve. I. The Relation of the Discharge from Single End Organs to Arterial Blood Pressure. J. Cell. and Comp. Physiol., **1:** 113, 1932.
23. Bronk, D. W., and Stella, G.: The Response to Steady Pressures of Single End Organs in the Isolated Carotid Sinus. Am. J. Physiol., **110:** 708, 1935.
24. Koch, E., and Mies, H.: Chronischer arterieller Hochdruck durch experimentelle Dauerausschaltung der Blutdruckzügler. Krankheitiforsch., **7:** 241, 1929.
25. Koch, E.: Die Reflektorische Selbststeuerung des Kreislaufes. Ergebnisse der Kreislaufforschung. Band I, Steinkopff, Dresden, 1931.
26. Heymans, C., and Bouckaert, J. J.: Observations chez le chien en hypertension artérielle chronique et expérimentale. C. R. Soc. de biol., **106:** 471, 1931.
27. Heymans, C., and Bouckaert, J. J.: Hypertension artérielle chronique expérimentale et sympathectomie. Bull. Acad. Roy., Med. Belg. 6 Série, **1:** 42, 1936.
28. Thomas, C. B., and Warthin, T. A.: The Response of Normal Dogs and Dogs with Experimental Hypertension to a Standard Cold Stimulus. Am. Heart J., **19:** 316, 1940.

29. ALPERT, L. K., AND THOMAS, C. B.: Studies in Experimental Hypertension. II. The Effect of Dietary Protein on the Urea Clearance and Arterial Blood Pressure in Chronic Hypertension. Bull. Johns Hopkins Hosp., **72:** 274, 1943.

30. ALPERT, L. K., AND LILIENTHAL, J. L., JR.: Studies in Experimental Hypertension. III. The Effect of Dietary Protein on the Clearance of Diodrast and Inulin by the Kidney in Chronic Hypertension. Bull. Johns Hopkins Hosp., **72:** 286, 1943.

31. KREMER, M., WRIGHT, S., AND SCARFF, R. W.: Experimental Hypertension and the Arterial Lesions in the Rabbit. Brit. J. Exp. Path., **14:** 281, 1933.

32. BOYD, J. D., AND MCCULLOGH, G. P.: Experimental Hypertension following Carotico-Aortic Denervation in the Rabbit. Quart. J. Exp. Physiol., **27:** 293, 1938.

33. NOWAK, S. J. G.: Chronic Hypertension Produced by Carotid Sinus and Aortic-Depressor Nerve Section. Ann. Surg., **111:** 102, 1940.

34. GRIMSON, K. S.: The Sympathetic Nervous System in Neurogenic and Renal Hypertension. Experimental Correlation and Clinical Consideration. Arch. Surg., **43:** 284, 1941.

35. THOMAS, C. B.: Experimental Hypertension from Section of Moderator Nerves: Relationship to Presence of Kidney Tissue. Proc. Soc. Exp. Biol. and Med., **48:** 24, 1941.

36. KINARD, F. W., AND ERVE, VAN DE J.: Control of Mange in Laboratory Dogs. J. Lab. Clin. Med., **21:** 1203, 1936.

37. HAMILTON, W. F., BREWER, G., AND BROTMAN, I.: Pressure Pulse Contours in the Intact Animal. I. Analytical Description of a New High-Frequency Hypodermic Manometer with Illustrative Curves of Simultaneous Arterial and Intracardiac Pressures. Am. J. Physiol., **107:** 427, 1934.

38. KOLLS, A. C.: An Indirect Method for the Determination of Blood Pressure in the Unanesthetized Dog. J. Pharm. and Exp. Therap., **15:** 443, 1920.

39. KOLLS, A. C., AND CASH, J. R.: The Blood Pressures in the Unanesthetized Dog. Bull. Johns Hopkins Hosp., **34:** 49, 1923.

40. WOOD, J. E., JR., AND CASH, J. R.: Experimental Hypertension—Observations on Sustained Elevation of Systolic and Diastolic Blood Pressure in Dogs. J. Clin. Invest., **15:** 543, 1936.

41. GOLDBLATT, H.: Studies on Experimental Hypertension. VII. The Production of the Malignant Phase of Hypertension. J. Exp. Med., **67:** 809, 1938.

42. HOLMAN, D. V., AND PAGE, I. H.: The Cardiac Output in Arterial Hypertension. Part II. A Study of Arterial Hypertension Produced by Constricting the Renal Arteries in Unanesthetized and Anesthetized (Pentobarbital) Dogs. The Am. Heart J., **16:** 321, 1938.

43. AYMAN, D., AND GOLDSHINE, A. D.: Blood Pressure Determinations by Patients with Essential Hypertension. I. The Difference Between Clinic and Home Readings Before Treatment. Am. J. Med. Sci., **200:** 465, 1940.

ON THE ISOLATION AND PROPERTIES OF THE FLUORESCENT FACTOR F_2 FROM HUMAN URINE[1]

VICTOR A. NAJJAR, VIRGINIA WHITE AND DWIGHT B. McNAIR SCOTT

From the Department of Pediatrics, Johns Hopkins University, Baltimore, Md.

Submitted for publication December 30, 1943

In 1940, Najjar and Wood (1) described the presence in urine of a substance with a characteristic bluish fluorescence, the excretion of which was related to the availability of nicotinic acid, being increased in proportion to the nicotinic acid intake. Subsequent observations showed that in the urine of patients with pellagra (2) this substance, designated as F_2, was regularly absent, a finding confirmed by Sydenstricker and Singal (3). Observations on dogs with experimental nicotinic acid deficiency (4) showed that F_2 disappeared from the urine of these animals as the deficiency progressed and reappeared only when nicotinic acid was added to the diet. The relation to pellagra was further shown by the observation that of a series of pyridine compounds given to normal humans, only those possessing antipellagric action gave rise to the excretion of F_2 in the urine (5). Ellinger, Glock and Platt (6) have successfully employed the excretion of F_2 in experimental animals for the bioassay of nicotinic acid in diets. The excretion of F_2 has been employed with success for the diagnosis of nicotinic acid deficiency abroad (7) as well as in this country.

Our interest in the chemical nature of F_2 was considerably enhanced by the observation that concentrates of this substance possessed biological activity (8) and that it was present in a number of tissues (9), notably in liver. The desirability of isolating this substance in pure form for the further study of its physiological and biological effects was then obvious. The present report deals with our progress in this endeavor.

PROCEDURE FOR PURIFICATION OF F_2

Although we have demonstrated the presence of F_2 in the urine of a number of animal species—dogs, rats and pigs—human urine has

[1] This study was aided by grants from the Williams-Waterman Fund of the Research Corporation and from Mead Johnson and Company.

proved most convenient as a source of material. Urine was obtained from volunteers who ingested 0.5 grams nicotinamide;[2] the peak of F_2 excretion occurs within the first hour or two, but the urine was usually collected for a 12 hour period. The development of a purification procedure has been greatly facilitated by the ease with which F_2 can be recognized in a crude mixture by its characteristic fluorescence. With a little practice, it can be recognized by the naked eye without the necessity of spectrography at every step. It is, however, necessary to observe certain precautions to prevent contamination with other bluish fluorescent materials such as may be obtained from stopcock grease, rubber tubing, etc.

The steps in our purification procedure are the following: (1) a preliminary extraction of urine with adsorbent charcoal, which removes nearly all of the pigments and other substances but under appropriate conditions does not remove appreciable amounts of F_2; (2) adsorption of F_2 on permutit; (3) elution with $BaCl_2$; (4) extraction with isobutyl alcohol; (5) precipitation with ether and (6) subsequent crystallization from various solvents. These various steps will now be described in detail.

Separation from Urinary Pigments. The urine was preserved by the addition of 2% glacial acetic acid and kept on ice. Before extraction it was diluted with an equal volume of distilled water and the pH adjusted between 3.0 and 3.5 by the further addition of glacial acetic acid. "Decolorizing charcoal" (Mallinckrodt) was then added, 1 gm. per 100 cc. of diluted urine, and after shaking 15 to 30 seconds in a separatory funnel the charcoal was filtered off. The resulting filtrate should be clear and colorless unless the urine is more than 24 hours old, in which case it may exhibit a faint yellowish tinge. The filtrate contains all the F_2 of the original urine, and is completely free from thiamin and riboflavin. It contains at least one other fluorescent compound in addition to F_2, a compound giving a bluish violet fluorescence. Accurate adjustment of the pH before adsorption is a matter of importance, for at more alkaline pHs variable amounts of F_2 are adsorbed by the charcoal and some urinary pigment escapes adsorption.

Adsorption of F_2 on Permutit. The charcoal filtrate containing the

[2] Generously supplied by Merck & Co., Inc.

F_2 was passed through a column of activated permutit[3], 60–80 mesh. A column 6 inches high and 2.5 inches in diameter was used to adsorb 1 liter of filtrate; this contained approximately 300 gms. permutit. The rate of flow was controlled by an ungreased stopcock at the base of the column, being maintained at 3–4 cc. per minute.[4] When drainage ceased, the column was washed with 6 liters of distilled water heated to 70°–80° C.; the wash water was then completely sucked off.

Elution of F_2. F_2 can be eluted from the permutit column completely by saturated NaCl or KCl, less completely by saturated $BaCl_2$, and to a considerable extent by strong alkalies and acids; the last named both destroy considerable F_2. We have preferred to use $BaCl_2$ in spite of the somewhat less complete elution for the reason that certain contaminants are less freely eluted by this agent. 300 cc. of cold saturated $BaCl_2$ were poured over the permutit column, care being taken to keep the stopcock open until the fluid reached the bottom of the column. This was found important in eliminating the formation of air bubbles and canalization of the permutit, which may result in poor elution. This same precaution should be taken during adsorption. The rate of flow of the eluate from the column should not be allowed to exceed 1 cc. per minute.

The eluate so obtained should be water-clear and colorless. In addition to F_2, it was found to contain some of the bluish violet fluorescent material mentioned above, but decidedly less than the charcoal filtrate.

Extraction of F_2 from the Eluate. F_2 is most readily extracted by butyl alcohol but almost equally well by isobutyl alcohol. We have used the latter agent which is more selective and takes up less impurities. Before extraction the solution must be rendered alkaline, for only minimal quantities of F_2 are extracted from acid solutions. We have employed $Ba(OH)_2$ to make the solution alkaline.

[3] 300 gms. permutit (Decalso) are boiled in 500 cc. 25% KCl solution for 1 hour with constant stirring. The supernatant fluid is discarded. This procedure is repeated twice. The permutit is then washed in a Buchner funnel with 10 liters or more of hot water, until it is practically chloride-free. It is then dried in an oven at 80°–90°C. overnight.

[4] Some heat is always generated in the permutit during adsorption. We have also noted that the fluid draining from the column frequently exhibits a yellow color despite the fact that it was colorless when introduced into the column.

To 200 cc. $BaCl_2$ eluate an equal amount of fresh saturated $Ba(OH)_2$ was added, followed by 300 cc. redistilled (non-fluorescent) isobutyl alcohol. This was shaken vigorously for 10–15 minutes in a separatory funnel, after which the aqueous layer was separated and reextracted with 200 cc. isobutyl alcohol. The combined alcohol extract, which was turbid, was centrifuged until clear[5], and the clear supernatant fluid separated and washed 3 times with an equal volume of distilled water with vigorous shaking. The barium salts are removed by the water; there is also some loss of F_2 with each washing, but the bulk of the F_2 remains in the alcohol. The washed alcohol was then evaporated down in an atmosphere of nitrogen[6] to a small volume (5 cc.) in a Friedrichs condenser on a water bath at 60°–70° C.

The concentrated isobutyl extract, which again became turbid due to deposition of more barium salts, was now clarified by centrifugation. It was found to contain from 40% to 50% of the F_2 present in the original urine and, when kept on ice, this remained stable. The solution was yellow in color and showed an intense greenish blue fluorescence due to the presence of two chief fluorescent compounds—F_2 and another compound giving violet fluorescence. The separation of the F_2 from this contaminant was accomplished in the following manner:

The yellowish isobutyl concentrate was evaporated to dryness in a vacuum desiccator where it was allowed to remain for another 12 hours at reduced pressure. It was then taken up with 50 cc. water which dissolves the F_2 and only a fraction of the violet fluorescing material. Most of the latter remains behind as a dark brown oily substance. The water extract was transferred to a separatory funnel and extracted with 50 cc. ether; the latter takes up only traces of F_2 but removes the remaining violet fluorescing material completely. The ether extraction was repeated 3 or 4 times until the ether extract no longer exhibited violet fluorescence. The purified watery solution was then evaporated to dryness in vacuo under nitrogen.

Preparation of Crystalline F_2. The waxy material obtained on

[5] The sediment, originally formed on the addition of $Ba(OH)_2$, is somewhat brown in color; it is soluble in HCl, and the solution gives none of the reactions of F_2.

[6] In the presence of oxygen, F_2 under this condition undergoes oxidation, forming a compound with an intense violet fluorescence.

desiccation was taken up in redistilled isobutyl alcohol; 5 volumes of ether were then added slowly with constant stirring. The solution became turbid as the ether was being added, and was then set aside overnight in the ice box. On standing, a pale brown amorphous precipitate was apparent, a material which on testing was found to possess in high degree the fluorescence characteristics of F_2, whereas the mother liquor showed a corresponding reduction. The ether precipitate was washed several times with ether and was then taken up in various solvents for further study and crystallization.

FIG. 1. F_2 CRYSTALS FROM ETHYL ACETATE. MAGNIFICATION × 3

(a) Crystallization from ethyl acetate. A sample of the amorphous ether precipitate was taken up in hot ethyl acetate, in which it dissolved with some difficulty. The solution was then concentrated to half its volume and set aside in the ice box. In the course of several weeks' time, small crystals were noted; they were pale yellow in color, waxy in consistency and formed in needles which branched at times (Fig. 1). The crystals were strongly hygroscopic and exhibited the characteristic fluorescence of F_2. Their physical and chemical properties are further described below.

(b) Crystallization from water. A sample of the amorphous ether precipitate, dissolved in water and concentrated at room temperature under reduced pressure and allowed to stand in a desiccator, deposited

needles (Fig. 2) similar to those described above but larger and darker in color. The dark color may be attributed to the presence of water, for we have noted a marked darkening in color of the amorphous ether precipitate on taking up moisture.

(c) Crystallization from methyl alcohol. A similar sample of amorphous ether precipitate was dissolved in distilled methyl alcohol and left on ice. Minute needle-like crystals, pale brown in color, formed in clusters on the surface of the mother liquor. These too, were hygroscopic and turned darker on standing and taking up moisture. Their physical and chemical properties were identical with those of the foregoing crystalline preparations and the amorphous ether precipitate.

FIG. 2. F_2 CRYSTALS FROM WATER. MAGNIFICATION × 2

PHYSICAL AND CHEMICAL PROPERTIES OF CRYSTALLINE F_2

The appearance of the crystals has already been described. Solubility is marked in water, methyl, ethyl, butyl, isobutyl, and amyl alcohols; less so in octyl and propyl alcohols and in ethyl acetate and very slight in ether, petroleum ether, xylene, and toluene. Concentrated solutions exhibit a yellow to brown color, depending on the concentration. Because of the waxy nature of the crystals, an exact melting point was not obtainable.

Fluorescent Properties. The fluorescence of F_2 in ultraviolet light is affected by the reaction of the solution. The characteristic greenish blue fluorescence described in our earlier work (4) is obtained in neutral or weakly alkaline solutions. In strongly alkaline solutions a more greenish fluorescence is obtained, and in acid solutions a more bluish fluorescence. In more concentrated solutions of F_2 in the acid range, a bluish white fluorescence is seen. The material does not fluoresce

appreciably in daylight or in blue light. The fluorescence of aqueous solutions of F_2 is decreased as the concentration of electrolytes in the solutions is increased.

In butyl and isobutyl alcohol, when extracted from an alkaline aqueous medium, the fluorescence is more intense than that in the aqueous medium and on standing it increases, reaching a maximum. This same increase in fluorescence on standing was observed in our early work on the estimation of F_2 in urine (1).

Behavior Toward Adsorbents. The behavior with charcoal adsorbents depends to some extent upon the pH of the solution. At low pHs, F_2 is not appreciably adsorbed by Mallinckrodt's decolorizing charcoal, norite, nuchar "T" or nuchar "W", but is adsorbed to a limited extent by darco. In alkaline media, it appears to be adsorbed to a variable extent by all these charcoals.

With the earths, it is readily adsorbed by permutit and floridin except at high pHs where adsorption is incomplete. From these agents it is readily eluted by concentrated salt solutions, strong acids and alkalies as has already been described.

Electrophoretic Behavior. When an aqueous solution of F_2 is put in an electrophoresis cell, the F_2 moves to the cathode. This fact, like the adsorbability on permutit, bears witness to the basic nature of F_2.

Stability. F_2 is more stable in acid than in alkaline solutions; it deteriorates more rapidly at higher temperatures and on exposure to ultraviolet light. With strong alkalies, deterioration is accompanied by the development of a deeper yellow color, a change which is irreversible. The resulting product behaves like nicotinic acid towards cyanogen bromide, a property not possessed by the original F_2. It seems probable that a hydrolytic cleavage is brought about by the alkali. The formation of a complex reacting with cyanogen bromide indicates the close relation of F_2 to nicotinic acid. Hydrolysis can also be accomplished by strong acids with the development of a positive cyanogen bromide reaction.

Oxidation. Atmospheric oxygen causes a slow oxidation of F_2 at room temperature, a more rapid one as the temperature is increased; it is also oxidized by an alkaline solution of $K_3Fe(CN)_6$. In both instances, the resulting product exhibits a deep violet fluorescence quite different from that of F_2 and somewhat less intense.

Behavior with Nitrous Acid. Freshly generated nitrous acid abolishes the color and fluorescence of F_2 solutions instantly and irreversibly.

Behavior with Sulfanilic Acid. A solution of F_2 was treated with HNO_2 followed by the addition of a few drops of 5% sulfanilic acid and enough Na_2CO_3 to make the solution alkaline. A bright orange color developed which increased in intensity for a period of about one hour, but faded on standing overnight.

Behavior with Acetone. A small quantity of F_2 concentrate was rendered alkaline with strong KOH, the addition of acetone producing a bright yellow color which increased in intensity for a period of 1 to 2 hours. The resulting product, when extracted with butyl or isobutyl alcohol exhibited a yellowish green fluorescence with a virage similar to that given by solutions of uranyl salts and by uranium glass.

Behavior with Acetic Anhydride. On treating crystalline F_2 with acetic anhydride a product was obtained with the following properties. Extraction with butyl or isobutyl alcohol from acid solution yielded a product giving violet blue fluorescence. From alkaline solution extraction with these alcohols gave a product showing a greenish and stronger fluorescence.

THE CHEMICAL IDENTIFICATION OF F_2

That F_2 is a derivative of nicotinamide seemed probable from the close relation of its excretion to the dietary intake of that substance. The fact that alkaline hydrolysis of F_2 gives rise to a substance which gives a positive cyanogen bromide reaction supports this view. In our first report (1) a group of pyridine derivatives were studied in the hope that similarities in their properties with those of F_2 might provide a clue to the chemical identity of the latter. None of the 32 compounds studied could, however, be identified with F_2, nor could any of the following derivatives which were studied at a later date: cozymase, dehydrocozymase, desaminocozymase, nicotinamide nucleoside and acetyl nicotinamide.

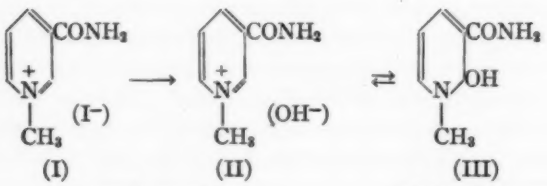
Some time ago our attention was attracted to the report of Karrer and his co-workers (10) who had studied reduction products of N-methyl nicotinamide and obtained compounds with properties similar in many respects to those of F_2. These authors prepared the pyridinium compound nicotinamide methiodide (I), a non-fluorescent

crystalline faintly yellow solid by treating nicotinamide with methyl iodide. On reduction with $Na_2S_2O_4$ oily, waxy compounds were obtained which were soluble in butanol and exhibited strong bluish fluorescence.

The similarity of Karrer's reduction products to our F_2 preparations led us to prepare and investigate some of these compounds. Following the technique of Karrer, N-methyl, N-ethyl and N-propyl reduced nicotinamides were prepared and their physical and chemical properties compared with that of F_2 (9). Of the three reduction products the N-methyl gave the strongest similarity to F_2 in all the physical and chemical reactions mentioned above. It also showed comparable values of fluorescence per unit of weight. The acetylated product, however, unlike that of F_2, maintained a violet-blue fluorescence both in acid and alkali media, and showed a different absorption spectrum from that prepared from F_2.

The further observation was then made that the change in the physical properties of the N-alkyl nicotinamides to waxy hygroscopic fluorescent products was effected *by alkali alone* regardless of the presence of any reducing agent.

We therefore attempted to identify the compound formed by alkali treatment of N-methyl nicotinamide iodide with F_2. Barium hydroxide was used for this purpose. Such alkalinization is known to effect a complete removal of the halide leaving a quaternary base (II) which is in large measure converted into a carbinol (III) with a shift in valences (11, 12).



N-methyl nicotinamide iodide, after treatment with $Ba(OH)_2$, was extracted with isobutanol and crystallized from methanol in the same manner as F_2. The physical and chemical properties of the product so obtained were found to correspond with those of F_2 so far as we have

observed the latter as described above. The absorption spectra (Fig. 3) of both F_2 and the N-methyl alpha-carbinol (III) obtained by treatment of the methiodide also correspond.[7]

It is clear then that urinary eluates and quaternary salts of N-methyl nicotinamide,[8] when treated with alkali and extracted with butyl alcohol, followed by evaporation and crystallization from methanol, yield products which are identical in physical and chemical characteristics. The final product in each case appears to be N-

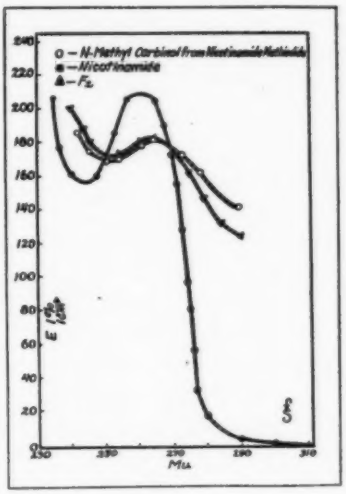


FIG. 3

methyl α-carbinol nicotinamide. However, despite the apparent identity of the final product, one may still question whether the urinary constituent which gives rise to the fluorescent reaction is actually a quaternary salt of N-methyl nicotinamide.

Huff and Perlzweig (13) on the basis of their studies concluded that such was the case and cited as evidence their observation that picrates obtained from urine eluate and from the chloride of N-methyl nico-

[7] We are indebted for these measurements to Dr. N. H. Coy of the Squibb Institute for Medical Research.

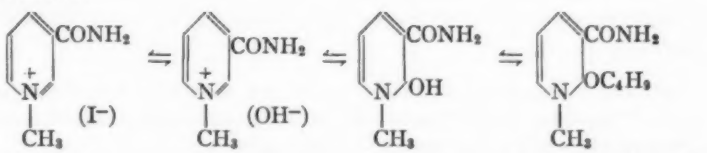
[8] We have studied the chloride and bromide, as well as the iodide.

tinamide had identical melting points (189.5°C). This observation we have confirmed and have further shown that halogen-free picrates, prepared from the chloride, bromide and iodide of N-methyl nicotinamide, give identical melting points, indicating that picric acid displaces the halide anion of the original compound. The identity of the picrates obtained from urine and from the quaternary pyridinium salts mentioned, as well as the evidence we have presented on the identity of the alkalinized products, leaves little room for doubt that the fluorogenic compound in urine is a derivative of N-methyl nicotinamide. Nevertheless, on the basis of available evidence we are not inclined to accept the conclusion of Huff and Perlzweig that the urinary ingredient is the quaternary ion N-methyl nicotinamide. The following evidence points to a different conclusion: Ellinger and Coulson (14, 15) although they, too, obtained an identical picrate from urine and from N-methyl nicotinamide iodide, prepared aurates from these two sources that differed in color and in melting point. Since picric acid is a relatively strong acid, it might well produce chemical changes other than the substitution of picrate for the halide anion, and might well convert some chemically related precursor into the pyridinium salt. Secondly, we have observed that the production of fluorescence on treatment with butanol and alkali takes place much more rapidly from urinary eluates than from the quaternary salts where equivalent fluorescent units were used, suggesting that time is required for the conversion of the latter into some compound which exists preformed in urine. Thirdly, we have found the quaternary pyridinium salts to be non-fluorescent, and if these represent the end product of nicotinamide metabolism in urine, one would expect that their ingestion would lead merely to their increased excretion without any increase in the bluish fluorescence of the *untreated* eluate. However, we find that the ingestion of N-methyl nicotinamide chloride regularly causes measurable increase in the bluish fluorescence of the untreated eluate—a phenomenon observed also with nicotinamide ingestion—which suggests that both these substances give rise to the excretion of a derivative which is not the simple pyridinium salt.

It is our view that the fluorogenic ingredient in urine is largely, if not entirely the α-carbinol of N-methyl nicotinamide (III) although it is possible that a small amount of pyridinium salt is present in

equilibrium with this compound. The formation of a quaternary picrate from urine may be explained by the fact that picric acid, being a relatively strong acid, forces the reversible reaction (II) $\rightleftarrows$ (III) to the left, leading to the formation of a quaternary salt from the carbinol, a reaction which the weaker auric acid is probably unable to accomplish. Existence of the carbinol preformed in urine would explain the more rapid development of fluorescence on alkalinization and butanol addition than occurs with the quaternary salt. The excretion of a slightly fluorescent material in untreated urine eluate similar in color and intensity to that of the carbinol nicotinamide, which we have observed after the administration of either nicotinamide or halides of N-methyl nicotinamide, is in agreement with this view. Further evidence of the identity of the carbinol and the material in untreated urinary eluate is now being sought.

The Chemical Nature of F_2 (the highly fluorescent compound). Studies of N-methyl pyridines as well as N-methyl quinolines and acridines (11, 12) have shown that with alkali treatment a quaternary pyridinium base is first formed, which by rearrangement, is in large part converted into a carbinol. Such carbinols combine readily with alcohols to form carbinol-ethers. In the case of nicotinamide methiodide, when treated with alkali and isobutanol, the reactions would be as follows:



Quaternary salt (non-fluorescent)	Quaternary base (non-fluorescent)	Carbinol (faintly fluorescent)	Carbinol-ether = F_2 (highly fluorescent)
(I)	(II)	(III)	(IV)

We were led to the conclusion that the carbinol from our urinary eluates must form a condensation product with the alcohol from the observation that minute additions of the butanol to the alkaline carbinol solution, enough to saturate the aqueous layer, but insufficient to cause a separation of an alcohol layer, produced nevertheless a very sudden and striking increase in fluorescence. The progressive forma-

tion of a carbinol-ether after an isobutanol extract has been made explains the hitherto puzzling increase in fluorescence on standing, a finding we reported in our earliest work (1). When the alcohol is evaporated to dryness this carbinol ether is apparently broken down leaving the carbinol itself; for when the dried product is dissolved in water, its fluorescence is found to be greatly diminished, and when extracted with alkali and butanol the marked increase in fluorescence again occurs.

SUMMARY

1. Observations are presented dealing with the isolation and properties of F_2, a bluish fluorescent substance obtained from urine when the body is adequately supplied with nicotinic acid.

2. The highly fluorescent compound F_2, obtained upon treating urinary eluates with alkali and butanol, is apparently derived from a precursor which shows only a slight bluish fluorescence. The procedure used—alkalinization and butanol extraction—serves to convert this precursor into the highly fluorescent compound F_2.

3. The identity of the urinary precursor is not yet completely established. The evidence at hand indicates it to be mostly N-methyl nicotinamide alpha-carbinol.

4. The highly fluorescent substance F_2, formed from the urinary precursor, appears to be a butyl ether of N-methyl nicotinamide alpha-carbinol.

We wish to express our indebtedness to Dr. Alsoph H. Corwin of the Department of Chemistry, Johns Hopkins University, for many helpful suggestions and criticisms which he has made during the course of this work.

BIBLIOGRAPHY

1. NAJJAR, V. A., AND WOOD, R. W.: Proc. Soc. Exp. Biol. and Med., **44:** 386, 1940.
2. NAJJAR, V. A., AND HOLT, L. E., JR.: Science, **93:** 20, 1941.
3. SYDENSTRICKER, V. P., AND SINGAL, S. A.: Proc. Vitamin Conference, Am. Assoc. Adv. Sci., Gibson Island, Md., Aug., 1941.
4. NAJJAR, V. A., STEIN, H. J., HOLT, L. E., JR., AND KABLER, C. V.: J. Clin. Invest., **21:** 263, 1942.
5. NAJJAR, V. A., AND HOLT, L. E., JR.: Proc. Soc. Exp. Biol. and Med., **48:** 413, 1941.

6. ELLINGER, P., GLOCK, G. E., AND PLATT, B. S.: Biochem. J., **36:** (Proc.) xi, 1942.
7. PLATT, B. S.: Personal Communication.
8. NAJJAR, V. A., AND HILL, J. H.: Unpublished observations.
9. NAJJAR, V. A., SCOTT, D. B. M., AND HOLT, L. E., JR.: Science, **97:** 537, 1943.
10. KARRER, P., SCHWARTZENBACH, G., BENZ, F., AND SOLLMSEN, U.: Helvet. Chim. Acta., **19:** 811, 1936.
11. DECKER, E., AND KAUFFMANN, A. J.: Prakt. Chem. (2), **84:** 432, 1911.
12. TAYLOR, AND BAKER: Sidgwick's "Organic Chemistry of Nitrogen" p. 524, Clarendon Press, Oxford, 1937.
13. HUFF, J. W. AND PERLZWEIG, W. A.: Science, **97:** 538, 1943; J. Biol. Chem., **150:** 395, 1943.
14. ELLINGER, P., AND COULSON, R. A.: Nature, **152:** 383, 1943.
15. COULSON, R. A., AND ELLINGER, P.: Biochem. Jour. **37:** (Proc.) xvii, 1943.

THE LABORATORY DIAGNOSIS OF NICOTINIC ACID DEFICIENCY. AN IMPROVED PROCEDURE FOR THE DETERMINATION OF F_2 (N-METHYL NICOTINAMIDE DERIVATIVE) IN URINE[1]

VICTOR A. NAJJAR

From the Harriet Lane Home, Johns Hopkins Hospital and the Department of Pediatrics, Johns Hopkins University, Baltimore, Md.

Submitted for publication June 1, 1944

Two laboratory procedures have been described for the detection of nicotinic acid deficiency. Najjar and Wood (1) described a bluish fluorescent substance in normal urine which was increased by the administration of nicotinic acid. This material, subsequently designated as F_2 (2) was found to be absent in pellagra, in which condition a load test with nicotinic acid or nicotinamide showed a marked reduction in the expected F_2 output. A second diagnostic procedure, first proposed by Field, Melnick, Robinson and Wilkinson (3) and confirmed by Perlzweig, Sarrett and Margolis (4) was based on a diminished excretion of trigonelline in the urine in pellagra, particularly after a load test with nicotinamide. The recent observations of Huff and Perlzweig (5) have made it clear that the trigonelline method used by the Michigan and Duke workers included the measurement of F_2, and Sarrett (6) has furthermore shown that most, if not all of the increased "trigonelline" in the urine after a load test was actually F_2. It was thus clear why the trigonelline determination showed the relation to nicotinic acid deficiency which had been observed; it measured the one metabolite which has been shown to vary consistently in this deficiency—namely, F_2.

The chemical nature of F_2 is discussed in an accompanying publication (7). It appears that the urinary ingredient which disappears in pellagra is secreted in the form of a precursor possessing little or no fluorescence. This precursor is converted by the addition of alkali

[1] This study was aided by grants from the Williams-Waterman Fund of the Research Corporation and from Mead Johnson & Co.

and butanol in the analytical procedure into a highly fluorescent compound. The highly fluorescent end product appears to be a butyl ether of N-methyl nicotinamide α-carbinol, but concerning the exact nature of the precursor there is disagreement. According to Huff and Perlzweig (5) it is the cation N-methyl nicotinamide, whereas Najjar and his collaborators (7) maintain that it is N-methyl nicotinamide alpha-carbinol. It is also possible that an equilibrium exists between these two forms, both of which are present in urine. Be this as it may, it is clear that either type of compound will, in the analytical procedure employed, be converted into an identical fluorescent end product, and either one may therefore be employed as a standard for the analytical procedure. Since N-methyl nicotinamide chloride is conveniently prepared and is less hygroscopic than the carbinol, we have chosen to use it as a standard of reference, expressing the urinary output of N-methyl derivative in terms of micrograms of N-methyl nicotinamide chloride, rather than in fluorescent units based on a quinine equivalent as we have done in the past.[2]

The purpose of the present communication is to describe an improved analytical procedure for the measurement of N-methyl nicotinamide derivatives in urine, which avoids certain difficulties encountered with our original method.

As now carried out the analytical procedure consists of the following steps: treatment with adsorbent charcoal, adsorption on permutit, elution with KCl, alkalinization of the eluate, extraction with butyl alcohol and measurement of the fluorescence of the butyl alcohol extract. The preliminary treatment of the urine with adsorbent charcoal was introduced in order to eliminate entirely errors introduced by the presence of urinary pigments and the fluorescent substance F_1 (2); this step also removes thiamin and riboflavin. The error introduced by the presence of other fluorescent pigments is of relatively little significance when load tests with nicotinamide are employed and the

[2] For the convenience of those who wish to compare older data expressed in units with present data in terms of grams of N-methyl nicotinamide chloride, it may be pointed out that one Najjar-Wood unit (defined as the amount of F_2 giving a fluorescence equal to that of 1 γ quinine sulfate when measured photometrically with the optical system used by Hennesy and Cerecedo (8) for thiamin determinations) is the equivalent of 35 γ N-methyl nicotinamide chloride.

quantity of F_2 is large, in which case charcoal adsorption may b omitted. In the case of casual urine specimens, however, the error may be considerable.

METHOD

A 25 cc. sample of fresh urine[3] is acidified with 1 cc. glacial acetic acid and 0.1 to 0.2 g. adsorbent charcoal[4] is added. The sample is stirred for a few seconds until the charcoal is thoroughly mixed and is then filtered through a rapid filter paper. 20 cc. of the clear or slightly yellowish filtrate are then passed through a glass adsorption column[5] containing 3–4 g. activated permutit.[6] The rate of flow through the column should not exceed 2 cc. per minute. The column is then washed with 40–60 cc. distilled water with suction. Care should be taken to give the tip of the column a careful rinsing with distilled water in order to remove any possible contaminating fluorescent substances derived from the rubber suction tube. The column is then eluted with 25% KCl. The rate of flow of the KCl solution from the column should not exceed 1 cc. per minute. 11 cc. of the eluate are collected,

[3] Urine may be preserved for analysis by the addition of 2 to 3 per cent of its volume of glacial acetic acid. The urine sample should contain no more than the equivalent of 50 γ N-methyl nicotinamide chloride. With higher concentrations, such as may occur after a load test, a smaller sample diluted to 25 cc. with 2% acetic acid should be used.

[4] The adsorbent charcoal supplied by Mallinckrodt Chemical Works, St. Louis, is excellent for this purpose. Satisfactory results are also obtained with Nuchar "T" and "W" supplied by West Virginia Pulp and Paper Co., Tyrone, Pennyslvania.

[5] Suitable adsorption columns can be purchased from E. Machlett & Sons, 220 East 23rd St., New York City or can be made from standard glass tubing 8–10 mm. in diameter, drawn to a tip at the base. A small packing of glass wool is introduced at the tip to keep the permutit in place.

[6] Activation of permutit is done in the following manner: 500 g. commercial permutit, 60–80 mesh (The Permutit Co., New York City) are boiled gently for 1 hour in a solution of 25% KCl containing 5% acetic acid. After draining off the fluid the process is repeated with a new lot of KCl and acetic acid. The permutit is then thoroughly washed with hot distilled water until it is almost chloride-free. It is then left to dry in an oven at about 100°C. Such permutit should not be used for more than a single determination. It may, however, be reactivated by boiling with KCl as above, after which it is tested for fluorescent substances by the usual elution procedure.

of which a 5 cc. aliquot is transferred into a glass stoppered tube or separatory funnel. 13 cc. of distilled n-butanol are then added followed by 1 cc. 15% NaOH, after which the container is immediately shaken vigorously for 2 minutes. This step is critical, since the necessarily strong alkali will destroy appreciable amounts of F_2 (methyl nicotinamide derivatives) if allowed to stand before shaking. Another 5 cc. aliquot of the eluate is treated similarly except that no alkali is added. This serves as a blank.

The samples are then centrifuged at low speed to separate the alcohol from the aqueous layer. The latter is drained off through a separatory funnel or pipetted off, and the butanol is cleared with a pinch of anhydrous sodium sulfate. The alcohol extracts are then allowed to stand for 20 minutes to develop the full fluorescence of the carbinol-ether compound, after which fluorescence is measured by means of a fluorophotometer[7] or visually in a fluorocomparator.

When determinations are made with a fluorocomparator, the butyl alcohol extracts may be matched against standards prepared from known concentrations of N-methyl nicotinamide chloride.[8] The comparisons should of course be made in similar tubes containing similar quantities of fluid. We have found that standards treated with alkali and extracted with butanol to convert the N-methyl nicotinamide chloride into the fluorescent carbinol-ether are stable when kept on ice for over ten days.

When using a fluorophotometer, the instrument must first be standardized as described below; simultaneous readings are then made of the blank and of the alkali treated sample. If A is the fluorescence (in galvanometer units) of the alkali treated sample, B the fluorescence of the blank from the urinary eluate untreated with alkali, M the fluorescence of the butanol extract of a solution containing 10 micrograms methyl nicotinamide chloride in 5 cc. 25% KCl, treated with

[7] We have found the Pfaltz and Bauer instrument satisfactory, using filters UG2 for the exciting light and BG14 and GG3 for the fluorescent light. We have also used the Coleman instrument equipped with a B-1 filter for the exciting light and PC-1 filter for the fluorescent light.

[8] N-methyl nicotinamide chloride may be purchased from W. A. Taylor & Co., 7300 York Road, Baltimore, or may be prepared by the method of Karrer et al. (9).

alkali and butanol exactly as is done with the urine eluate, and B′ the fluorescence of a butanol extract of pure KCl, then:

$$\frac{A - B}{M - B'} \times \frac{11}{5} \times \frac{26}{20} \quad \text{or}$$

$$\frac{A - B}{M - B'} \times 28.6 = \gamma \text{ methyl nicotinamide chloride in the original 25 cc. urine sample.}$$

STANDARDIZATION OF THE FLUOROPHOTOMETER

Before making fluorescence measurements the instrument should be turned on for fifteen minutes with all filters in place to allow the source of light to reach a stable maximum. It is not necessary to use N-methyl nicotinamide chloride for standardization each time the instrument is used, and it is usually more convenient to employ a quinine solution for that purpose. Once the quinine equivalent has been determined for a standard solution of N-methyl nicotinamide chloride with a particular optical system, the quinine can be substituted for the daily standardization. In practice we employ a solution containing 20 γ quinine sulfate in 0.1 N H_2SO_4 for the routine setting of the instrument. For the original standardization the methyl nicotinamide butanol extract is first measured in the cuvette and the diaphragm or the amplifier of the instrument adjusted until it registers some arbitrary round number in galvanometer units. The quinine standard is then substituted and the reading in galvanometer units recorded. On subsequent occasions when the quinine standard is used, adjustment of the diaphragm or amplifier to reproduce this same galvanometer reading will reproduce the optical conditions obtained with the N-methyl nicotinamide chloride standard.

VALIDITY OF THE PHOTOMETRIC DETERMINATION

In order to establish the validity of the photometric procedure it is necessary to show that under a given set of optical conditions, the fluorescence of the carbinol ether is directly proportional to the concentration of N-methyl nicotinamide chloride originally present. Data are presented in fig. 1 which demonstrate the linear relationship between the concentration of N-methyl nicotinamide chloride or urinary eluate and the fluorescence of the carbinol ether derived from it over a range of concentration between 10γ and 40γ at a constant setting of the instrument.

It is also of interest to know whether the intensity of fluorescence obtained from the carbinol ether is directly proportional to the exciting light in the range of concentration studied, since upon occasion it may be necessary to work with exciting lights of reduced intensity (low current; aging ultraviolet bulbs) conditions which it may be impossible to compensate for by a wider opening of the diaphragm or increase in amplification. We have demonstrated such a linear relationship over a wide range of concentration in the case of the carbinol ether which

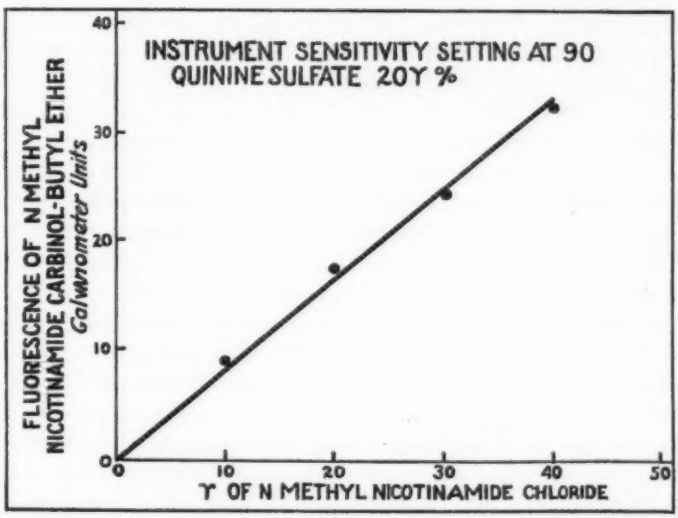


FIG. 1. shows the linear relationship between the concentration of N-methyl nicotinamide chloride or urinary eluate and the fluorescence of the carbinol-butyl ether derived therefrom. (Measurements made with Coleman instrument.)

permits one to work with variations in the adjustment of the fluorophotometer when the source of light becomes feeble.

SIGNIFICANCE OF THE F_2 (N-METHYL NICOTINAMIDE DERIVATIVE) DETERMINATIONS FOR THE DIAGNOSIS OF NICOTINIC ACID DEFICIENCY

For the past three years the writer has employed the technique described above for the estimation of F_2 in normal individuals, in those

with suspected nicotinic acid deficiency and in a few instances of definite clinical deficiency. Excretions have been carried out on "fasting hour" (10) specimens and oral and intravenous load tests have also been used. Space will not be taken here to present the data obtained in detail, but merely to summarize them.

With the original technique described for the measurement of F_2, the presence of contaminating fluorescent substances tended to mask small quantities of F_2 present in fasting hour or in casual specimens, and occasionally even zero values were encountered which could not be related to nicotinic acid deficiency. With the improved charcoal adsorption technique described in the present communication, which we have employed during the past 3 years, we have failed to encounter such false negatives, and have found zero excretions only in the few cases of clinical pellegra that we have had the opportunity to examine.

It may also be of interest to record the fact that casual urine specimens are more useful in the diagnosis of nicotinic acid deficiency than those obtained after periods of fasting, for we have frequently observed that fever, starvation and other conditions accompanied by acute wasting tend to cause a temporary increase in F_2 output.

Although, as we have pointed out above, the casual urine specimen has not failed to reveal the presence of frank pellagra, it is clear that various grades of nicotinic acid deficiency are more clearly defined by means of load tests in which nicotinamide is given, either orally or parenterally. Such load tests reveal differences in the severity of pellagra, as was pointed out in our original publication on the subject (2); they will also reveal different degrees of depletion that have not yet given rise to clinical symptoms. In using the oral load test we have given 100 mgms. nicotinamide to adults. With this dosage normal individuals have excreted between 2100 and 4000 γ N-methyl nicotinamide chloride in 4 hours. We are inclined to think that excretions below the former figure may be significant of depletion. Further experience is, however, necessary before drawing conclusions on this point. Somewhat larger excretion figures are obtained with parenteral load tests.

SUMMARY

1. A modification of the original Najjar-Wood procedure for the estimation of the fluoroscent factor F_2 (an N-methyl nicotinamide

derivative) in urine is described. This eliminates interference from certain other fluorescent substances and increases the sensitivity and accuracy of the test.

2. It is proposed to standardize F_2 measurements in terms of N-methyl nicotinamide chloride, rather than in terms of arbitrary quinine units.

3. The significance of this test procedure in the diagnosis of nicotinic acid deficiency is briefly discussed.

BIBLIOGRAPHY

1. Najjar, V. A., and Wood, R. W.: Proc. Soc. Exp. Biol. and Med., **44:** 386, 1940
2. Najjar, V. A., and Holt, L. E., Jr.: Science, **93:** 20, 1941.
3. Field, H., Jr., Melnick, D., Robinson, W. D. and Wilkinson, C. F., Jr., J. Clin. Invest. **20:** 379, 1941.
4. Perlzweig, W. A., Sarrett, H. P., and Margolis, L. H.: J. A. M. A., **118:** 28, 1942.
5. Huff, J. W., and Perlzweig, W. A.: J. Biol. Chem., **150:** 395, 1943.
6. Sarrett, H. P.: J. Biol. Chem., **150:** 395, 1943.
7. Najjar, V. A., White, V., and Scott, D. B. M.: Bull. Johns Hopkins Hosp., **74:** 378, 1944.
8. Hennessy, D. J., and Cerecedo, L. J.: J. Am. Chem. Soc., **61:** 179, 1939.
9. Karrer, P., Schwarzenbach, G., Benz, F., and Solmssen, U.: Helv. Chim. Acta, **19:** 826, 1936.
10. Holt, L. E., Jr. and Najjar, V. A.: Journal-Lancet, **63:** 366, 1943.

A CASE OF PELLAGRA DEVELOPING ON A HOSPITAL WARD IN A PATIENT RECEIVING VITAMIN B COMPLEX

DEAN W. ROBERTS AND VICTOR A. NAJJAR

From the Harriet Lane Home, Johns Hopkins Hospital and the Department of Pediatrics, Johns Hopkins University, Baltimore, Md.

Apart from certain areas of the Southern United States, where it may be encountered in families in the lower economic brackets, pellagra in children is certainly a rare disease. During the past 30 years only six cases have been recognized among more than 130,000 admissions to the wards and dispensary of the Harriet Lane Home. The present case is of unusual interest, not only because of its rarity but because it developed in a child who had been under medical observation for eight weeks, who was taking a well balanced diet with the addition of vitamin B complex and in whom possible predisposing factors, although present, were by no means obvious.

CASE HISTORY

L. B. (H. L. H. #A29646) a twelve year old white girl was admitted to the Harriet Lane Home on April 28, 1943 with a complaint of anorexia, fatigue and gradual loss of weight of more than a year's duration.

The family history was, on the whole, irrelevant. The financial circumstances were comfortable. Although a broken home afforded a certain basis for nervous tension, this did not appear to play a part in the picture of somatic disease which developed.

The past history included attacks of measles, pertussis and varicella—all before the age of eight years, none of them severe. There were a moderate number of upper respiratory infections, and, at the age of four, an attack of purulent otitis media which became chronic, following which tonsillectomy and adenoidectomy were performed and, subsequently, a simple and then a radical mastoidectomy, the latter at the age of six years. The ear had given no trouble for the three years preceding the present illness.

The present illness began insidiously in the spring of 1942 with fatigue, anorexia and loss of weight. The patient was sent to Florida for several months, but showed no conspicuous improvement. In August of that year, just before returning from the south, an eruption developed that was diagnosed as erythema nodosum. Red and tender indurated nodules the size of beans appeared over one ankle and on the shins and there was a spiking fever of 101° to 104°F. The fever and lumps disappeared in the course of 10 days but the patient failed to improve.

She was hospitalized for study for 5 weeks in the Children's Hospital in Washington, but the only findings of consequence were a sinus tachycardia (pulse 135), a moderate hypochromic anemia (hemoglobin 9.5 gms.) and an elevated sedimentation rate. Cardiac consultations were said to rule out rheumatic fever. Studies of kidney function were normal.

Between August 1942 and April 1943 the patient's weight decreased from 78 lbs. to 60 lbs. The most conspicuous symptom during this period was anorexia. Although offered a liberal diet containing cereal, eggs, bacon, green vegetables, meat, potatoes and a quart of milk daily, only small amounts of the quantity offered were taken. The patient was frequently nauseated and at times there was vomiting after meals. At times the stools were somewhat loose—from two to five a day—, but this was not constant; they were never noted to contain pus or blood. Intermittently there was a low grade fever. She continued to go to school somewhat irregularly during the winter of 1942–1943, but was easily fatigued and showed a tendency to drowsiness.

On admission the patient was found to be an emaciated girl of somewhat linear body build, without evidences of puberty. She was intelligent and cooperative. There was no evidence of any discomfort. The skin was somewhat sallow, but showed no lesions of note. The extremities were somewhat cold. The mucous membranes showed moderate pallor. Her hair was abundant and dry, there was no general glandular enlargement, and the thyroid was not palpable. Eyes, nose and mouth were essentially normal. The tongue was normal in appearance. The right ear drum was absent and there was a mastoidectomy scar behind right ear. The left ear was normal. The chest was long and thin. The lungs were normal to percussion and auscultation. The heart rate was 136/min. in spite of the fact that the child did not seem excited. The heart was normal in size to percussion and sounds were of fair quality. At the left sternal border, fourth interspace, there was a short systolic murmur which was not transmitted. This murmur was believed to be functional. Blood pressure was 82/62. The abdomen was essentially negative; the liver and spleen could not be felt. There was no tenderness to palpation and no masses were made out. Genitalia were normal and there was no discharge. The extremities were long and thin; there was no swelling of joints and no tenderness or local heat. Tendon reflexes were equal and active; there was no ankle clonus and Kernig and Babinski tests were negative.

Laboratory examinations on admission showed hemoglobin to be 9.7 gms., r.b.c. 4,560,000, volume of packed cells 34.2, w.b.c. 10,650, corrected sedimentation rate 22. Differential white count was: Juvenile neutrophiles 14%; Seg. neutrophiles 65%, lymphocytes 14%, monocytes 7%. The smear showed numerous platelets, moderate achromia, slight microcytosis and moderate poikilocytosis. There were no nucleated red cells. Icterus index was 6, the mean corpuscular volume 75, the mean corpuscular hemoglobin 21 and mean corpuscular hemoglobin concentration 28.

Urine—specific gravity 1.030; negative for albumin and sugar; microscopic

essentially normal. The tuberculin test was negative to 1.0 mgm. and the serological test for syphilis was negative.

Following admission to the hospital the patient continued a downward course. At times there would be periods of several days in which she was afebrile, to be followed by irregular febrile episodes in which the temperature would rise as high as 102° to 103° F. and on one occasion reached 104.8° F. The tachycardia was conspicuous, the pulse remaining over 120, even during the absence of fever. Numerous studies undertaken failed to throw light on the nature of the illness. Repeated blood cultures were sterile and agglutinations for typhoid, paratyphoid, various dysentery organisms, suipestifer, enteritidis, tularense, several strains of brucella and proteus X_2 and X_{19} showed no increased agglutinin titers. There was no increased heterophile antibody titer. A nose and throat consultation exonerated the ear and mastoid as a possible focus of infection. Cultures of the nasopharynx for pneumococci were negative. X-ray of the chest showed normal lung fields and a heart of normal size and contour. The electrocardiogram was normal except for sinus tachycardia. X-rays of the bones and gastrointestinal tract were negative. Stool cultures were negative for pathogenic organisms and no parasites or ova were discovered. Kidney function studies were normal and studies of the urinary tract, including urine cultures and pyelography, failed to disclose any pathological condition. Therapy consisted in a high caloric diet supplemented with orange juice and a liquid extract of yeast, given in doses of 4 cc. per day, to furnish the B complex. The hemoglobin fell to 8 grams, but responded well to a transfusion. In spite of the supportive measures the patient continued to lose weight gradually.

Eight weeks after admission it was noticed that a symmetrical brown pigmentation had developed on the backs of the hands, extending up to the knuckles where it was sharply demarcated from the pale skin beyond. (Figs. A and B) Brown pigmented areas were likewise noted over the callouses of the elbows. The suspicion of pellagra was borne out by the appearance of the tongue which was red, notably at the margins, and showed atrophy of the papillae. The diagnosis was confirmed by studies of the excretion of the fluorescent factor F_2 (N-methyl nicotinamide derivative) in urine (1). A casual urine specimen showed complete absence of F_2 and even after a test dose of 100 mgms. of nicotinamide, no trace of F_2 appeared in the urine. Careful questioning of the patient at this time indicated that she had for some time past had intestinal symptoms of which she had not complained to the doctors or nurses. These consisted of mild diarrhea and occasional abdominal cramps. As the patient was ambulant and had lavatory privileges, the presence and persistence of the diarrhea was not appreciated.

Some light upon the pathogenesis of the pellagra was obtained from a consideration of the vitamin content of the yeast extract which had been used in treating the patient. It was found that the daily dose which the patient had been getting contained 0.3 mg. thiamin and 10.0 mgs. riboflavin, but only insignificant amounts of nicotinic acid. It therefore seemed likely that, in the presence of an obscure

FIGS. A (upper left) and B (lower left). *Appearance of Tongue and Hands before Therapy.* Note red, swollen tongue with atrophy of papillae, and pigmentation on the backs of the hands.
FIGS. C (upper right) and D (lower right). *Normal Appearance of Tongue and Hands 2 Months after Nicotinamide Therapy.*

intestinal disorder which had been causing interference with intestinal absorption over a prolonged period, the small amount of nicotinic acid ingested was totally inadequate.

Following the diagnosis of pellagra the patient was treated with nicotinamide (100 mgms. q.d. by mouth). Improvement in the appearance of the tongue was striking within 48 hours. The pigmentation cleared more slowly, but had disappeared entirely two months later (Figs. C and D).

The patient as a whole, however, did not respond in any striking way to the treatment of the pellagra and the gastrointestinal symptoms became more pronounced, confirming the impression that these were the cause rather than a manifestation of the pellagra. The diarrhea became more severe; microscopic blood was frequently found in the stools and on one occasion there was gross blood. Pus, too, was found in the stools and umbilical pain was more pronounced. No evidence of ulcerative colitis was obtained at this time by proctoscopy or barium enema, but barium studies of the small intestine were highly suggestive of regional ileitis. The patient was placed on sulfasuxidine, which caused no improvement. Sulfaguanidine was then substituted for this, but this drug, too, was without apparent effect. It was, nevertheless, continued throughout the stay in the hospital.

The possibility of abdominal exploration was considered, but was not thought advisable because of the patient's poor general condition and particularly because of increasing evidence of damage to the heart. At the time the pellagra was recognized, it was noted that the quality of the heart sounds had become poor and some three or four weeks later there developed evidences of frank cardiac failure—an enlarged heart with gallop rhythm, edema of the lower extremities and an enlarged liver. The electrocardiogram at this time showed a biphasic P_{II}, an inverted P_{III}, very low voltage QRS complex in all leads and an inverted T_{III}. Anemia could be excluded as a cause of cardiac failure, since the hemoglobin had been maintained by repeated small transfusions. Digitalis therapy was instituted and at the same time liver extract was given intramuscularly and a vitamin B concentrate intravenously. There was gradual improvement in the condition of the heart within the next two weeks, evidences of failure disappearing. Nevertheless enlargement persisted and even a month later there was an episode of gallop rhythm following slight exertion.

Following the episode of cardiac failure—at which time the prognosis seemed very poor—there was a very gradual improvement in the patient's general condition. Her weight—58 lbs. after the disappearance of the edema—increased to 72 lbs. over a period of 2½ months. Her appetite improved and she became afebrile. The diarrhea gradually decreased and stools containing gross blood or pus were only occasionally noted; six weeks after the cardiac failure episode the bowel movements became normal. The electrocardiographic changes, too, disappeared and the patient was discharged from the hospital, having shown a steady improvement in strength and general appearance, on December 19, 1943. Subse-

quent reports since discharge indicate a continuous, though gradual improvement in general condition.

COMMENT

From what has been said above, it seems clear enough that an obscure intestinal disorder, perhaps of the nature of a regional ileitis, interfered with intestinal absorption to an extent sufficient to cause pellagra. Evidences of other B deficiencies were apparently prevented by the use of a supplement which contained thiamin and riboflavin, but only minimal quantities of nicotinic acid. The cause of the cardiac failure remains obscure. The possibility that this might be due to beriberi was considered, but such a diagnosis was not supported by studies of urinary thiamin excretion, and in any event would be difficult to reconcile with the therapy being given at the time. Other diagnostic possibilities considered were disseminated lupus erythematosus and periarteritis nodosa, but supportive evidence for these could not be obtained and the cause of the cardiac failure still remains obscure.

The chief interest in the case lies in the fact that the precipitating agent of the pellagra—the diarrhea—was so well concealed at the time the child was under observation. It is also pertinent as an illustration that commercial yeast extracts cannot be relied on as sources of nicotinic acid.

SUMMARY

A case of pellagra is described which developed in a 12 year old girl who was under hospital observation and was receiving a "B complex" preparation at the time.

It is pointed out that certain commercial yeast extracts do not supply sufficient quantities of nicotinic acid.

BIBLIOGRAPHY

1. NAJJAR, V. A.: The Laboratory Diagnosis of Nicotinic Acid Deficiency. An Improved Procedure for the Determination of F_2 (N-Methyl Nicotinamide Derivative) in Urine. Bull. Johns Hopkins Hosp., **74**: 392, 1944.

THE ANTI-BLACK TONGUE ACTIVITY OF N-METHYL-NICOTINAMIDE CHLORIDE[1]

VICTOR A. NAJJAR, MARGARET M. HAMMOND, MARY ALLEN ENGLISH, MARIA B. WOODEN AND CAROLYN C. DEAL

From the Harriet Lane Home, Johns Hopkins Hospital and the Department of Pediatrics, Johns Hopkins University

Submitted for publication June 3, 1944

In 1940, the presence of a previously unrecognized nicotinic acid derivative in human urine was reported (1). It was found to increase markedly after the administration of nicotinic acid. This substance, later designated F_2, was noted to be absent in the urines of patients with pellagra (2) and of dogs with experimental nicotinic acid deficiency (3). It was further shown that only pyridine compounds with antipellagra activity produced an increased output of this substance in urine (4). Measurements of F_2 in urine, particularly after a load test of nicotinic acid or the amide, gave a clear indication of the extent of deficiency, both in pellagra and black tongue (3).

We were prompted to investigate the biological activity of this substance, in view of the possibility that it might be the active anti-pellagra principle in the body, for the following reasons: (a) the anti-pellagra action of the active pyridine compounds is always associated with the production of F_2; (b) measurement of the urinary output of F_2 showed a definite correlation with the state of deficiency of the organism; (c) F_2 was found in measurable quantities in body tissues, particularly the liver. We proceeded to measure its effect on the growth of microorganisms using F_2 crystals obtained by crystallization from methanol. It was found that in a synthetic asparagin-dextrose medium, F_2 in a concentration of 20 γ/cc. produced a ten-fold increase in the growth of E. coli, as compared to the control, after an eight hour incubation period. When the concentration of F_2 was increased to 100 γ/cc. a seventeen-fold increase of growth was obtained.[2] It was further observed that certain strains of H. influenzae and streptococcus, which failed to grow

[1] Aided by grants from Mead Johnson and Company, and the Williams-Waterman Fund of the Research Corporation.

[2] These observations were made in collaboration with Justina H. Hill.

on ordinary media, responded with luxuriant growth upon the addition of F_2 crystals[3] to the culture media.

Recent work has shown that the F_2 produced by nicotinamide ingestion is an N-methylnicotinamide compound (5, 6, 7, 8). The chloride of this substance can be prepared (9) easily and in large quantities. Encouraged by these preliminary observations, it was decided to test the activity of this compound[4] on nicotinic acid deficiency in dogs.

EXPERIMENTAL

Five young female dogs and one small adult male were used for this experiment. The animals received unlimited quantities of a black tongue diet which consisted of:

	Per cent
Yellow corn	65
Cow peas	8
Vitamin-free casein	7.5
Sucrose	7
Cotton seed oil	5
Cod liver oil	5
NaCl	1
$CaCO_3$	1.5

This diet furnished an intake of 1.5 γ thiamin and 1.0 γ riboflavin per gram of diet. Each dog showed an average urinary output of 80–150 γ thiamin and 130–280 γ riboflavin per day. The dogs were placed in metabolism cages and complete daily urine collections were obtained.[5] Measurements of F_2 in the urine (10) were made daily at

[3] These observations were made by Helen D. Zepp.

[4] The N-methylnicotinamide chloride used in these studies was prepared by Miss Virginia White. N-methylnicotinamide iodide, prepared by Karrer's method (9) was shaken with a suspension of silver chloride, and the resulting N-methylnicotinamide chloride was obtained by evaporation of the resulting filtrate and subsequent crystallization from methyl alcohol. It had a melting point of 237°C. (uncorrected).

The purity of the material was further checked for contamination with nicotinamide by converting it into a picrate; the resulting picrate melted at 189°C.

[5] Urine was collected in brown bottles containing glacial acetic acid, representing roughly 3% of the daily urinary volume. Preliminary adsorption of urine on charcoal was omitted from the procedure for F_2 determination because it was found not to be necessary in the case of dog's urine.

first, and, subsequently, every other day. The results were expressed in terms of N-methylnicotinamide chloride.

Three of the dogs (I, II, and III), all females, were put on the black tongue producing diet unsupplemented. The other three (IV, V, and VI), the last being a small adult male, were given in addition 50 mg. of N-methylnicotinamide chloride daily in oral capsules for twenty-four days. The dose was later reduced to 25 mg. daily for the rest of the experiment. All the dogs were carefully observed every day and weighed twice weekly.

RESULTS

Of the group supplemented with N-methylnicotinamide chloride (Fig. 1-A), the two young dogs (IV and V) showed a steady growth and gain in weight throughout the experiment. Dog VI definitely maintained its weight. Being a fully mature adult animal it could not be expected to gain much. All three animals in this group were active, lively and maintained a good appetite throughout the experiment. No vomiting, diarrhea or mouth lesions were observed. In fact, all three remained perfectly normal and symptom-free for over four weeks after all the unsupplemented dogs exhibited frank manifestations of black tongue.

Of the second group, which was receiving the same black tongue producing diet unsupplemented with N-methylnicotinamide chloride, all three dogs developed unmistakable signs and symptoms of the deficiency. Figure 1-B shows the persistent loss in weight two to three weeks after the start of the diet. Dog II, the first to develop symptoms, showed a marked and rapid loss in weight, particularly during the last two weeks. The animal looked sick during this period, then became apathetic and unresponsive. Diarrhea appeared and became rapidly worse, the stools soon becoming watery and blood-tinged. Vomiting became persistent and food, as well as water, was refused. It became too weak to walk or even stand and later seemed completely unresponsive. It was then obvious that the dog was dying. Subcutaneous and intraperitoneal saline was administered and 100 mg. of N-methylnicotinamide chloride was given orally, but the dog died a few hours later. Dog III presented exactly the same severe manifestations and was allowed to become moribund before the drug was

administered. Although there seemed to be a definite improvement in the animal's behavior and responsiveness a few hours after therapy,

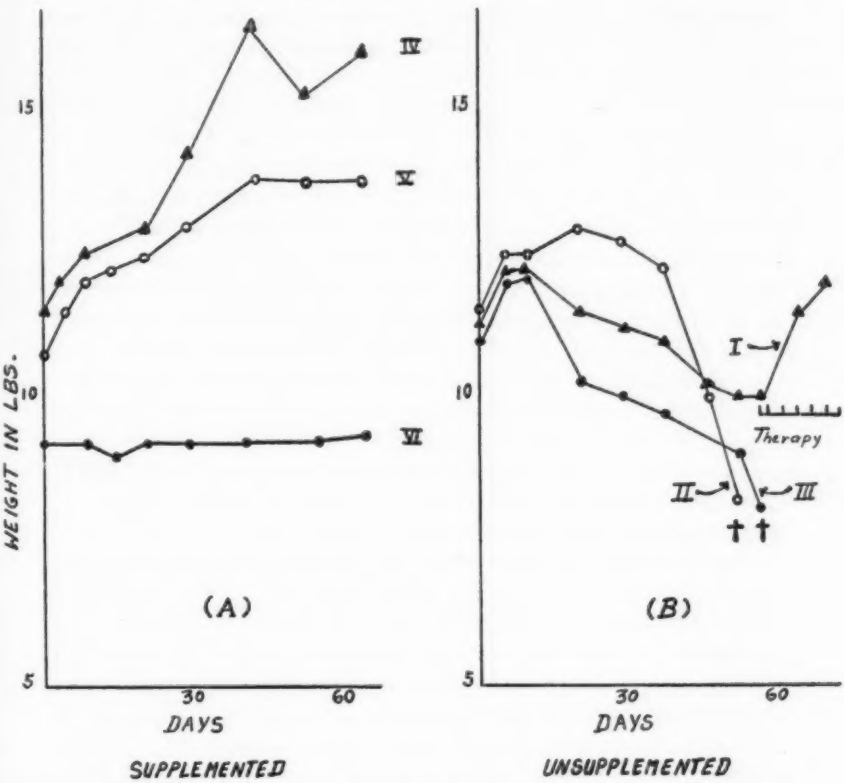


FIG. 1. (A) Weight curves of dogs receiving a black tongue producing diet supplemented with N-methylnicotinamide chloride, 50 mg. daily for twenty-four days and 25 mg. thereafter. Dogs IV and V were young females, while dog VI was a small fully grown adult male.

(B) Weight curves of dogs on a similar diet unsupplemented. † indicates death of the animal.

the diarrhea and vomiting persisted and death occurred twelve hours later. Autopsy of both dogs showed, grossly, an acute gastroenteritis

with ulceration of the buccal mucous membrane and discoloration of the tongue and palate. Heart, lungs, liver and kidneys were normal.

It was our intention to allow these two dogs to develop a maximal clinical picture of the deficiency. For this reason therapy was intentionally withheld until their condition became critical, and when it was given it was with the full realization that such treatment was probably too late to be effective. Our previous experience, as well as that of others, has shown that drugs known to be potent may be ineffective if given too late. In the case of the third dog (I), we decided to administer the drug as soon as diarrhea and vomiting appeared. Like the previous two, this dog lost considerable weight, became sluggish, refused food, but maintained a certain amount of water intake. Diarrhea then appeared which, though blood-tinged, was not as severe as in the preceding two dogs. There was no vomiting until the second day of the diarrhea. 100 mg. of N-methylnicotinamide chloride were then given orally. There was a marked improvement in three hours. The dog became active, responsive, ran around and started eating. A second dose of 150 mg. was given twelve hours later. Improvement continued with marked appetite for food and water. 50 mg. daily were given thereafter. In four days there was a weight gain of 1.5 pounds and this continued subsequently (Fig. 1-B).

The excretion of F_2, expressed as N-methylnicotinamide chloride, in dogs IV, V, and VI (Fig. 2-A) averaged about 350 γ daily during the control period on stock diet before the experimental diet was started. On the back tongue diet supplemented with the drug the excretion went up, as would be expected; it reached approximately 5,000 γ daily and fluctuated around this value. After the dose was reduced to 25 mg., the output of F_2 did not drop immediately, but declined gradually for about sixteen days after which it was maintained at an average level of about 1200 γ daily. This is an impressive demonstration of the ability of the animal to store the compound (see Fig. 2).

In the unsupplemented dogs the excretion of F_2, expressed as N-methylnicotinamide chloride, dropped gradually in all three dogs until it reached the zero level. The rapidity of the decline of F_2 output (Fig. 2-B) showed an excellent correlation with the subsequent development of symptoms. The sharpest decline in excretion, as well as the earliest attainment of a zero level, was obtained in

dog II which was the first to show symptoms. The decline was least sharp and the zero level was reached latest in dog I, which was the last to develop symptoms of deficiency. It is of interest to note that in the three dogs the zero level was reached eight to fourteen days before the onset of severe symptoms.

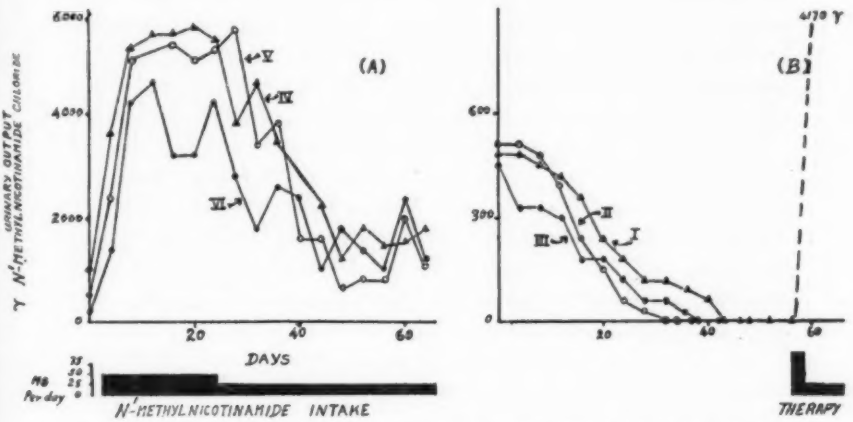


FIG. 2. (A) F_2 excretion per day, expressed as N-methylnicotinamide chloride, of the supplemented group receiving black tongue producing diet plus 50 mg. daily for twenty-four days and 25 mg. thereafter.

(B) F_2 excretion of the unsupplemented group receiving the deficient diet alone.

DISCUSSION

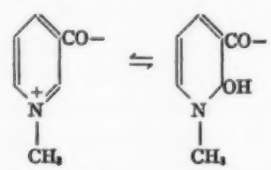
We have presented what we believe to be clear evidence that N-methylnicotinamide chloride possesses definite anti-black tongue activity, both as a preventive and as a therapeutic agent. The prophylactic approach in our experiment was planned because of the realization that in black tongue the gastroenteritis and dehydration may often be too far advanced for successful therapy. This was clearly exemplified in our failure to save dogs II and III in contrast to the success that attended early treatment of dog I. Woolley et al. (11), while studying the therapeutic effect of numerous pyridine derivatives on black tongue, failed to obtain a beneficial effect with nicotinamide methochloride (N-methylnicotinamide chloride). This failure, we believe, was very probably due to late administration of the drug.

The continuous gain in weight, the persistently normal behavior and the absence of any symptoms or signs of black tongue in dogs IV, V, and VI that received supplements of N-methylnicotinamide chloride is in sharp contrast with the loss in weight and the appearance of severe symptoms of black tongue in the group that received no such supplements (Dogs I, II, and III). When the drug was used therapeutically reasonably early, as in dog I, the symptoms disappeared immediately and a rapid gain in weight followed. These facts present inescapable evidence of the efficacy of this compound, the nucleus of which is a normal product of nicotinamide metabolism in the body.

At this point we are strongly inclined to advance the view that the F_2 (N-methyl pyridine β-carboxy-) nucleus[6] is the active group that prevents and cures black tongue. This impression is gained from the following facts: (a) Nicotinic acid, nicotinamide, sodium nicotinate, nicotinuric acid and coramine (the diethylamide of nicotinic acid) have all been shown to possess anti-black tongue activity (11). The same compounds after ingestion have also been shown to produce F_2 in urine (4). (b) In contrast to the above, quinolinic acid, dinicotinic acid and trigonelline are not effective compounds and do not produce

[6] It is certain, at the present time, that the F_2 obtained from urine following the administration of nicotinamide retains the carboxylamide group after it is methylated in the body at the ring nitrogen. We are by no means certain, however, that the other active drugs that get similarly methylated to produce F_2 and have a substituted amide or an ester linkage are hydrolyzed in the body to the amide or the acid, although that may well be the case. There is also no proof, to our knowledge, that the body can form the amide from nicotinic acid. On the contrary, we have considerable evidence which will be published shortly that such is not the case. We have found that the F_2 resulting from the administration of nicotinic acid, though similarly methylated, nevertheless does not acquire the amide linkage (14).

Because of the foregoing, we feel that the F_2 structure



(N-methyl pyridine β carboxy—) is the grouping that is essential for the activity of those compounds, regardless of the radical that attaches to the carboxy group.

F_2. (c) F_2 has been found in body tissues, particularly the liver (12). It has also been shown that liver slices in vitro can methylate nicotinamide to produce the N-methylnicotinamide compound (F_2) (13). (d) The urinary output of F_2 is a direct measure of the body stores of the antipellagra factor. (e) After the ingestion of nicotinamide, the main excretion product is an N-methylnicotinamide (5). (f) N-methylnicotinamide compounds are active compounds capable of reversible reduction (9) in a way similar to body coenzymes. It was for that purpose that Karrer synthesized them. (g) Finally, we have shown a compound containing this grouping to be active in stimulating the growth of certain bacteria and effective in preventing, as well as curing, black tongue (dog pellagra). We have every reason to expect a similar effect in preventing and curing human pellagra, although we have not as yet had the occasion to put this to the test.

Notwithstanding the evidence presented above, we are fully aware of the difficulty of proving conclusively to our own satisfaction the complete validity of this view. By the same token, there is no valid support to the concept that nicotinic acid itself is the antipellagra vitamin, beyond evidences that seem no more convincing than those we have presented in favor of N-methyl derivative. In fact, the lack of correlation between deficiency and the nicotinic acid output in the urine and the definite correlation with F_2 excretion speaks more in support of our view. It remains to be seen whether by inhibiting or exhausting the methylating mechanism of the body, nicotinamide would still be effective. If not, whether the already methylated N-methylnicotinamide compound would be active. Conversely, it is possible, but not probable, that the body can demethylate the (F_2) N-methylnicotinamide compound and thereby obtain available nicotinamide. It is obvious that the demonstration that one of the two drugs is more potent than the other in preventing and curing black tongue should offer conclusive proof.

CONCLUSION

1. N-methylnicotinamide chloride has been shown to be effective in preventing, as well as curing, black tongue in dogs. Its possible value in human pellagra is suggested.

2. The suggestion is advanced that the F_2 nucleus (N-methyl pyridine β-carboxy-) may be the active antipellagra vitamin.

BIBLIOGRAPHY

(1) NAJJAR, V. A., AND WOOD, R. W.: Proc. Soc. Exp. Biol. and Med. **44:** 386, 1940.
(2) NAJJAR, V. A., AND HOLT, L. E., JR.: Science, **93:** 20, 1941.
(3) NAJJAR, V. A., STEIN, H. J., HOLT, L. E., JR., AND KABLER, C. V.: J. Clin. Invest. **21:** 263, 1942.
(4) NAJJAR, V. A., AND HOLT, L. E., JR.: Proc. Soc. Exp. Biol. and Med. **48:** 413, 1941.
(5) HUFF, J. W., AND PERLZWEIG, W. A.: Science, **97:** 538, 1943.
(6) HUFF, J. W., AND PERLZWEIG, W. A.: J. Biol. Chem. **150:** 395, 1943.
(7) NAJJAR, V. A., AND WHITE, V.: Science, **99:** 284, 1944.
(8) NAJJAR, V. A., WHITE, V., AND SCOTT, D. B. M.: Bull. Johns Hopkins Hosp. **74:** 378, 1944.
(9) KARRER, P., SCHWARTZENBACH, G., BENZ, F., AND SOLLMSEN, U.: Helv. Chim. Acta. **19:** 811, 1936.
(10) NAJJAR, V. A.: Bull. Johns Hopkins Hosp. **74:** 392, 1944.
(11) WOOLLEY, D. W., STRONG, F. M., MADDEN, R. J., AND ELVEHJEM, C. A.: J. Biol. Chem. **124:** 715, 1938.
(12) NAJJAR, V. A., SCOTT, D. B. M., AND HOLT, L. E., JR.: Science, **97:** 537, 1943.
(13) PERLZWEIG, W. A., BERNHEIM, M. L. C., AND BERNHEIM, F.: J. Biol. Chem. **150:** 401, 1943.
(14) NAJJAR, V. A., AND WHITE, V.: Unpublished observations.

PROCEEDINGS OF THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

HELD IN HURD HALL, MAY 15, 1944

DR. ALFRED BLALOCK, PRESIDING

1. Polyarticular Arthritis and Osteomyelitis Due to Granuloma Inguinale. DRS. J. LYFORD III, R. B. SCOTT AND R. W. JOHNSON, JR.

SUMMARY

This paper was published in the March issue of the Bulletin of the Johns Hopkins Hospital, **74:** 213, 1944.

DISCUSSION

Dr. J. E. Moore opened the discussion by saying that granuloma inguinale, heretofore thought of as a local disease, now must be considered as a potential systemic disease. He congratulated Drs. Lyford, Scott and Johnson on a beautiful demonstration of this syndrome.

Dr. R. W. Johnson, Jr. said that the work was done largely by Drs. Lyford and Scott with a little coaching from the sidelines. This work has revealed a new type of arthritis caused by a disease hitherto considered local and apparently spread by the blood stream to widely disseminated joints. The most exciting part has been the demonstration of the causative agent in the test tube as well as the patient. Specific groups of arthritides have been split off the non-specific group of rheumatoid arthritis, and perhaps a number of such cases may now be related to diseases such as granuloma inguinale.

Dr. C. W. Wainwright thought this a most admirable demonstration of metastatic arthritis from granuloma inguinale. The organism has an affinity, not only for synovia, but also for bone. He wondered whether the arthritis might not be an extension from a nearby osteomyelitis.

Dr. G. O. Gey said that he had made many tissue cultures from rheumatoid arthritis, all negative save these. Many pleuro-pneumonia-like organisms have been followed for as long as 6 years, but there seemed to be peculiar difficulties in maintaining the organism of granuloma inguinale in tissue culture, for reasons not altogether clear.

Dr. R. B. Scott thought that biopsy led to good diagnostic results in these cases and that it should certainly be done in doubtful ulcerative lesions of the female genitalia as there is no evidence of a bad effect on the course of carcinoma in this region after biopsy.

2. Wiring and Electrothermic Coagulation of Aneurysms. Dr. Arthur H. Blakemore

SUMMARY

Dr. Blakemore started the work on aneurysms in the hope of alleviating the symptoms and perhaps also prolonging the life of a group of patients for whom little medical help was available. The aim of the treatment which he developed was to produce a firm blood clot which would reinforce the wall of the aneurysm and prevent its further extension. It was found that a suitable clot could be produced locally by the application of heat. A fine insulated wire was introduced and heated by means of an electrical current. Great care was necessary to avoid local overheating or improper placement of the wire. A special coiling device overcame both of these dangers by producing uniform and controlled distribution of the wire. Electrical measurements were made during the heating to determine the temperature of the wire. By observing the amount of current necessary to heat the wire to the desired temperature it was possible to estimate the blood flow about the wire. In this way the progress of clotting about the wire could be followed. X-rays of a number of patients were shown with excellent development of a clot in the desired position. The patients uniformly experienced relief of pain and in many cases the progressive enlargement of the aneurysm was halted. In some cases a second operation was necessary. The average duration of life after treatment was apparently prolonged and many patients were able to return to work. Ill effects following the treatment were uncommon and in several cases were due to certain factors inadequately controlled in the early trial of the method.

At Dr. Blalock's suggestion, Dr. Blakemore described a new method of repairing severed arteries without the use of sutures. Although this method is now only in an experimental stage the results are very encouraging.

DISCUSSION

Dr. J. E. Moore said that the medical treatment of saccular aneurysm has interested him and his co-workers. Their patients have been followed from the date of their first symptom or of the diagnosis of asymptomatic aneurysm. It is very difficult to judge the effects of treatments on one particular case because of the variable course. The first symptom may be sudden death, or an old aneurysm may clot and become static.

The average duration of life in the untreated cases was 15 months. With some treatment this period was doubled, with relief of symptoms. If treatment was adequate, life was prolonged to 70 months. All except 1 are now dead. Dr. Moore further stated that Dr. Blakemore had dated his results from operation, rather than from the first symptom which makes them look less good. His results, however, appear very favorable to Dr. Moore, who hoped that similar work will be followed here.

Dr. J. C. Owings thought that Dr. Blakemore had done a splendid piece of work, outstanding in this field. He hoped to start similar work here, but obstacles have been encountered in collecting suitable equipment. Dr. Blakemore's apparatus, he thought, was complex and difficult to move about, while his own, he hoped, would be a very much simpler apparatus. This has not yet been tried, but it is hoped to get under way soon.

Dr. A. H. Blakemore said that he was much encouraged by Dr. Owings' work and very enthusiastic about technical developments. Perhaps an apparatus could be developed so simple that a surgeon could run it without technical assistance.

Dr. Blakemore was very reticent about saying too much about this method of treatment until many more cases have been seen and a longer time has elapsed. Many cases have been treated as a last resort, but a few early cases are now coming in.

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal.)

Synopsis of Materia Medica, Toxicology, and Pharmacology (Third Edition). By FORREST RAMON DAVISON. 759 pp. Illus. $6.50. C. V. Mosby Company, St. Louis, 1944.

This work contains a chapter for each of the general topics of materia medica, prescription writing, and toxicology; eighteen chapters are devoted to the pharmacological actions and therapeutic uses of specific classes of drugs. The toxicology of an individual drug is usually considered in conjunction with the action and use of the drug.

Although there are a number of errors, most of the material presented is modern and useful. As in most books of this type, the documentation is poor, and it is quite difficult to locate the original source of the material from the few chapter references that are given. For this reason, the book cannot be recommended without restriction as a text for students, although it should be useful to those desiring a summary of the principal actions and uses of drugs.

A. C. B., Jr.

Manometric Methods. By MALCOLM DIXON. 2nd Edition, 155 pp. Illus. $1.75. The Macmillan Company, New York, 1943.

This excellent handbook on the manometric methods in general use for tissue respiration studies has been brought up to date in a new edition. The presentation is simple and direct. The author has succeeded in bringing out out the essential points of each method wihout undue distraction by technical detail. Adequate theoretical background and notes on the limitations of the methods are presented. At the end, there are sample calculations of data and some useful tables. This small book should be of great value both to the laboratory worker interested in tissue respiration studies and to any scientist who wishes to learn the working details and interpretation of the methods involved.

J. A. L.

The Hospital in Modern Society. By A. C. BACHMEYER and G. HARTMAN. 768 pp. $5.00. The Commonwealth Fund, New York, 1943.

One hundred forty-five selected and representative articles and publications relating to hospital administration are here published in one volume of twenty-nine chapters. Each chapter deals with a separate phase of the field and the titles run from "Plant Maintenance" and "Medical Records" to "Group Hospital and Health Insurance" and "Public Health". The work of ninety-eight authors from hospital

and allied spheres is included. Two medical administrators, Dr. Joseph C. Doane and the late Dr. S. S. Goldwater, are represented by fourteen articles.

This extensive coverage of the literature will be of great value not only to workers in the field, but also to students of medical administration. Although the articles come from literature written before 1941 and thus do not include discussion of recent social and wartime changes, the volume is enhanced by the extensive references for further reading, covering the literature up to July 1943, which are at the end of each chapter.

The editors are to be congratulated for the care and judgment exercised in the selection and editing of the materials included in this important addition to the few existing volumes concerning hospital administration.

E. L. C.

An Outline of General Physiology. 2nd Edition. By L. V. Heilbrunn. 748 pp. Illus. $6.00. W. B. Saunders Company, Philadelphia & London, 1943.

In the first chapter of this excellent book, general physiology is defined as "the science which deals with all forms of living material." It attempts to answer the question "What is life?" "The general physiologist believes that all life has something in common; his search is often for the least common denominator of living processes." This concept is very similar to that of Sir William Maddox Bayliss, who in his "Principles of General Physiology" defined general physiology as "the study of the endowment of living material." Heilbrunn's book is a masterful presentation of the wide field covered by this definition. After a brief survey of the statics of living systems the kinetics are treated at greater length. The power of living things to react in special and peculiar ways to stimulation is considered to be an outstanding characteristic of living things. This characteristic known as irritability is discussed comprehensively. This discussion is followed by a chapter on the theories of stimulation and anesthesia which are presented with great lucidity. Here, as in most chapters of this book, the reader is referred not only to the most recent articles on the subject, but to older, often half-forgotten and neglected monographs and articles. The student for whom this book is primarily intended will find it extremely useful as a basis for his future studies in biology or medicine. The advanced worker is presented with a guide to rich sources of information, many of which lie off the beaten track. The book is warmly recommended to everybody interested in the basic phenomena of life.

W. F.

On the Influence of Trades, Professions, and Occupations in the United States, in the Production of Disease. By Benjamin W. McCready, 1837. With an Introductory Essay by Genevieve Miller, 129 pp. $1.75. The Johns Hopkins Press, Baltimore, 1943.

This essay was submitted in competition for a prize offered by the Medical Society of the State of New York. Written in 1837 before the work of Pasteur

and Koch and almost a century before any occupational death rates were available, it presents a most interesting picture of the ideas current in those days concerning the effects of social-economic, environmental and occupational factors on disease and longevity among the various occupational classes, inluding the maritime, agricultural, manufacturing, trade and professional groups. The essay gives a picture of the habits of the workmen, the living and working conditions and the related health problems of the 19th century judged from the personal experience of a young doctor and the little published material available. McCready's suggestions for the correction of the existing conditions forecast many of the present day public health trends.

Miss Genevieve Miller, in the introduction, reviews the background of the essay and summarizes the principal points. Altogether it is a delightful book.

A. M. B.

The Jews and Medicine. Essays. By HARRY FRIEDENWALD, M.D. (With a foreword by HENRY SIGERIST) 2 Volumes. 772 pp. $7.50. Johns Hopkins University Press, Baltimore, 1944.

A delightful collection of essays about Jewish physicians of ancient and medieval times, the work of a humanist and a scholar, these volumes will be warmly welcomed by all who have an interest in medical history.

R. T. H.

The Johns Hopkins Hospital and the Johns Hopkins University School of Medicine: A Chronicle. Vol. I. By ALAN M. CHESNEY (With a foreword by WILLIAM H. HOWELL) 318 pp. $3.00. The Johns Hopkins Press, Baltimore, 1943.

Dean Chesney's account of the birth and early days of the Johns Hopkins Hospital and Medical School has replaced legend by a definitive history—well documented, but nevertheless highly readable. The original ideas of Johns Hopkins himself, the wise planning of President Gilman and John Shaw Billings, the part played by Miss Garrett are all clearly set forth as well as the many difficulties that were encountered in launching the project. One finds interesting accounts of the circumstances leading to the selection of the big four—Welch, Osler, Halsted and Kelly—and their associates Mall, Abel and Howell. Despite the limitations of space, the author has managed to picture the personalities and background of the leading actors in the scene—from the founder to the first medical faculty, and many excellent photographs have been included.

Dr. Chesney's book will be warmly welcomed by all who have been connected with Johns Hopkins and will be appreciated by many others who are interested in the development of American Medicine.

L. E. H.

Standard Methods for Examination of Dairy Products. 8th Edition. $3.00. American Public Health Association, 1941.

This edition contains nearly one hundred more pages than the previous edition. There is little revision of the old material but considerable new material on Micro-

biological Methods for the Examination of Frozen Desserts and Ingredients of Frozen Desserts, which now constitutes Part II. The ingredients include evaporated milk, condensed milk, dry milk, coloring solutions, flavoring extracts, fruit, nuts, sweetening agents and egg products. In the past it has been so common to confine suspicion to fluid milk that the inclusion of these other food products is a welcome advance. Tests for Sterility of Dairy Containers, Utensils and Equipment have also been much elaborated.

Although methods are described for examining milk for coliform organisms, streptococci, tubercle bacilli and *Brucella spp.*, little or no attention is given to staphylococci. Since outbreaks of staphylococcus food poisoning attributed to milk products are being reported with increasing frequency and since the staphylococcus toxin is not destroyed by pasteurization, this subject would seem to merit consideration.

The research worker, inclined to be an individualist, interested in obtaining results by the best possible methods and not averse to changing them in accordance with his own judgement, is likely to be impatient with any standardization of methods. On the other hand, public health work requires a certain amount of uniformity for purposes of law enforcement and so that results obtained in different laboratories may be reasonably comparable; hence the necessity for "standard methods". They should be so interpreted. The successive editions of this book satisfy this purpose admirably.

J. H. B.

Abdominal and Genito-Urinary Injuries. Prepared under the Auspices of the Committee on Surgery of the Division of Medical Sciences of the National Research Council. 243 pp. Illus. $3.00. W. B. Saunders Company, Philadelphia & London, 1942. (Military Surgical Manuals, Vol. 3.)

This manual which has been prepared under the auspices of the Committee on Surgery of the Division of Medical Sciences of the National Research Council is one of six volumes in a series concerned with the principles of military surgery. Its purpose is to make readily available to all medical officers reliable information regarding the peculiarities of war injuries and the general management of the patient, before, during, and after operation.

The volume consists of two parts, as indicated in the title. The first part presents in a remarkably succinct and compact manner the principles of abdominal surgery as applied specifically to war injuries. The eleven short chapters include consideration of the disposition of casualties, the mechanism of wound production, shock, hemorrhage, infection, diagnosis, general care, operative procedure, postoperative treatment, and the management of complications. The second part is devoted to genito-urinary injuries. Written in the same authoritative terse style, its six chapters deal first with a general survey of the diagnosis of war injuries, and then with a detailed consideration of the etiology, diagnosis, and treatment of injuries involving the kidney, ureter, bladder, and the external and internal

genitalia. The chapter on the care of the neurogenic bladder resulting from trauma to the nervous system is a classic.

Each chapter of the manual is written by one or more competent authorities with wide experience. Stress is placed on multiplicity of injuries, and the surgical approach which is recommended is the one best suited for all. For the sake of simplicity and compactness the authors have been obliged to eliminate debatable points, and to recommend one standard procedure. It therefore is not difficult to detect here and there evidence of personal preference and prejudice. In such a comprehensive work, however, it is surprising not that this occurs at all, but that it occurs so seldom.

H. J. J.

INDEX TO VOLUME LXXIV

Pagination According to Months:

January, 1944, pp. 1–84
February, 1944, pp. 85–160
March, 1944, pp. 161–228
April, 1944, pp. 229–274
May, 1944, pp. 275–319
June, 1944, pp. 321–422

Adrenal Cortical Damage, A Peculiar Type of, Associated with Acute Infections, and Its Possible Relation to Circulatory Collapse. Rich, Arnold R. 1

Albanese, Anthony A., Holt, L. Emmett, Jr., Brumback, Joseph E., Jr., Frankston, Jane E. and Irby, Virginia: Observations on a Diet Deficient in Both Methionine and Cystine in Man 308

Albanese, Anthony A., Holt, L. Emmett, Jr., Frankston, Jane E. and Irby, Virginia: Observations on a Histidine Deficient Diet in Man 251

Amino Acids, Influence on Histamine Reactions and Anaphylactic Reactions in Intestinal Strips of Guinea Pigs and in Intact Guinea Pigs. Landau, S. Walter and Gay, Leslie N. 55

Arthritis, Granuloma Inguinale as a Cause of. Report of a Case. Scott, Roger R., Lyford, John, III and Johnson, Robert W., Jr. 213

Bing, R. J.: The Effect of Hemoglobin and Related Pigments on Renal Functions of the Normal and Acidotic Dog 161

Blackman, Samuel S., Thomas, Caroline B. and Howard, John Eager: Effect of Testosterone on the Blood Pressure and Tissues of Rats 321

Black Tongue, Activity of N′-Methylnicotinamide Chloride (F_2 Chloride). Against Najjar, Victor A., Hammond, Margaret and English, Mary Allen. 406

Book Reviews 77, 156, 225, 270, 316, 418

Brooke, Wallace, and Day, Robert: Immunization with the Vole Acid Fast Bacillus Against Experimental Tuberculosis 275

Brumback, Joseph E., Jr. (See Albanese, Anthony A.) 308

Chickenpox, Congenital, with Disseminated Visceral Lesions. Oppenheimer, Ella H. 240

Cystine, Observations on a Diet Deficient in Both Methionine and, in Man. Albanese, Anthony A., Holt, L. Emmett, Jr., Brumback, Joseph E., Frankston, Jane E. and Irby, Virginia 308

Day, Robert, M.D. (See Brooke, Wallace) 275

Electrocardiographic Abnormalities, The Significance of, in Young Adults. Thomas, Caroline B. 229

English, Mary Allen (See Najjar, Victor A.) 406

Fatty Liver, Sudden Death in Young Adults in Association with. Graham, Robert Lee ... 16
Favism. Josephs, Hugh W. ... 295
Frerichs, John B.: The Influence of a Monocytosis of the Peripheral Blood Stream upon the Cellular Character of an Acute Inflammation ... 49
Fibrosis of the Lungs, Acute Diffuse Interstitial. Hamman, Louis and Rich, Arnold R. ... 177
Fluorescent Factor F_2, From Human Urine, Isolation and Properties. Najjar, Victor A., White, Virginia, and Scott, Dwight B. McNair ... 378
Frankston, Jane E. (See Albanese, Anthony A.) ... 251, 308

Gay, Leslie N. (See Landau, S. Walter) ... 55
Graham, Robert Lee: Sudden Death in Young Adults in Association with Fatty Liver ... 16
Granuloma Inguinale as a Cause of Arthritis and Osteomyelitis. Report of a Case. Scott, Roger B., Lyford, John, III and Johnson, Robert W., Jr., M.D. ... 213

Hamman, Louis and Rich, Arnold R.: Acute Diffuse Interstitial Fibrosis of the Lungs ... 177
Hammond, Margaret (See Najjar, Victor A.) ... 406
Hemoglobin and Related Pigments, The Effect of, on Renal Functions of the Normal and Acidotic Dog. Bing, R. J. ... 161
Histidine Deficient Diet in Man, Observations on. Albanese, Anthony A., Holt, L. Emmett, Jr., Frankston, Jane E. and Irby, Virginia ... 251
Holt, L. Emmett, Jr. (See Albanese, Anthony A.) ... 251, 308
Holt, L. Emmett, Jr. and Kajdi, Charlotte N.: The Nutritional Requirements in Inanition. I. Observations on the Ability of Single Foodstuffs to Prolong Survival ... 121
Holt, L. Emmett, Jr. and Kajdi, Charlotte N.: The Nutritional Requirements in Inanition. II. The Effect of Mineral and Vitamin Supplements on the Survival of Animals on Single Foods ... 142
Howard, John Eager (See Blackman, Samuel S.) ... 321
Hypertension, Experimental, from Section of Moderator Nerves. Thomas, Caroline Bedell ... 335

Inanition, Nutritional Requirements in. I. Observations on the Ability of Single Foodstuffs to Prolong Survival. Holt, L. Emmett, Jr. and Kajdi, Charlotte N. ... 121
Inanition, Nutritional Requirements in. II. The Effect of Mineral and Vitamin Supplements on the Survival of Animals on Single Foods. Holt, L. Emmett, Jr. and Kajdi, Charlotte N. ... 142
Irby, Virginia (See Albanese, Anthony A.) ... 251, 308

Johnson, Robert W., Jr. (See Scott, Roger B.) ... 213
Josephs, Hugh W.: Favism ... 295

Kajdi, Charlotte N. (See Holt, L. Emmett, Jr.) ... 121, 142

Landau, S. Walter and Gay, Leslie N.: Influence of Certain Amino Acids on Histamine Reactions and Anaphylactic Reactions in Intestinal Strips of Guinea Pigs and in Intact Guinea Pigs 55
Lee, Ferdinand C., Macht, David I. and Pierpont, Ross Z.: A Preliminary Report on a Method for Lengthening the Effect of a Sympathetic Nerve Block 119
Leftwich, William B.: An Intradermal Test for the Recognition of Hypersensitivity to the Sulfonamide Drugs 26
Lungs, Acute Diffuse Interstitial Fibrosis of. Hamman, Louis and Rich, Arnold R... 177
Lyford, John, III. (See Scott, Roger B., M.D.) 213

Macht, David I. (See Lee, Ferdinand C.) 119
Mackenzie, C. G. (See Mackenzie, Julia B.) 85
Mackenzie, Julia B. and Mackenzie, C. G.: Effect of Prolonged and Intermittent Sulfonamide Feeding on the Basal Metabolic Rate, Thyroid and Pituitary 85
Methionine and Cystine in Man, Observations on a Diet Deficient in. Albanese, Anthony A., Holt, L. Emmett, Jr., Brumback, Joseph E., Jr., Frankston, Jane E. and Irby, Virginia 308
Meyer, Eugene, and Meyer, Mary B.: The Pathology of Staphylococcus Abscesses in Vitamin C-Deficient Guinea Pigs 98
Monocytosis of the Peripheral Blood Stream, Influence of, upon the Cellular Character of an Acute Inflammation. Frerichs, John B. 49

Najjar, Victor A., Hammond, Margaret and English, Mary Allen: The Antiblack Tongue Activity of N′-Methylnicotinamide Chloride (F_2 Chloride) 406
Najjar, Victor A.: The Laboratory Diagnosis of Nicotinic Acid Deficiency. An Improved Procedure for the Determination of F_2 (N-Methyl Nicotinamide Derivative) in Urine 392
Najjar, Victor A. (See Roberts, Dean W.) 400
Najjar, Victor A., White, Virginia and Scott, Dwight B. McNair: On the Isolation and Properties of the Fluorescent Factor F_2 from Human Urine 378
Nicotinamide Chloride, N′-Methyl, The Antiblack Tongue Activity of. Najjar, Victor A., Hammond, Margaret and English, Mary Allen 406
Nicotinic Acid Deficiency, the Laboratory Diagnosis of. An Improved Procedure for the Determination of F_2 (N-Methyl Nicotinamide Derivative) in Urine 392
Nutritional Requirements in Inanition. I. Observations on the Ability of Single Foodstuffs to Prolong Survival. Holt, L. Emmett, Jr. and Kajdi, Charlotte N... 121
Nutritional Requirements in Inanition. II. The Effect of Mineral and Vitamin Supplements on the Survival of Animals on Single Foods. Holt, L. Emmett, Jr. and Kajdi, Charlotte N. 142

Observations on a Histidine Deficient Diet in Man. Albanese, Anthony A., Holt, L. Emmett, Jr., Frankston, Jane E. and Irby, Virginia 251
Oppenheimer, Ella H.: Congenital Chickenpox with Disseminated Visceral Lesions.. 240

Pellagra, A Case of, Developing on a Hospital Ward in a Patient Receiving Vitamin B. Complex. Roberts, Dean W. and Najjar, Victor A. 400
Pierpont, Ross Z. (See Lee, Ferdinand C.) 119

Proceedings of the Meeting of the Johns Hopkins Medical Society..152, 218, 266, 313, 415
Public Health, Proper Attention to the Role of Emotional Social Factors in Illness as a New Step in. Robinson, G. Canby..... 259

Rich, Arnold R.: A Peculiar Type of Adrenal Cortical Damage Associated with Acute Infections, and Its Possible Relation to Circulatory Collapse..... 1
Rich, Arnold R. (See Hamman, Louis)..... 177
Roberts, Dean W. and Najjar, Victor A.: A Case of Pellagra Developing on a Hospital Ward in a Patient Receiving Vitamin B Complex..... 400
Robinson, G. Canby: Proper Attention to the Role of Emotional and Social Factors in Illness as a New Step in Public Health 259

Scott, Dwight B. McNair (See Najjar, Victor A.)..... 378
Scott, Roger B., Lyford, John, III and Johnson, Robert W., Jr.: Granuloma Inguinale as a Cause of Arthritis and Osteomyelitis. Report of a Case..... 213
Sulfapyrazine, Experimental Studies on, In Mice. Trevett, Gordon I..... 299
Sulfonamide Drugs, An Intradermal Test for the Recognition of Hypersensitivity to the. Leftwich, William B..... 26
Sulfonamide Feeding, Effect of Prolonged and Intermittent, on the Basal Metabolic Rate, Thyroid and Pituitary, Mackenzie, Julia B. and Mackenzie, C. G..... 85
Sympathetic Nerve Block, A Preliminary Report on a Method for Lengthening the Effect of. Lee, Ferdinand C., Macht, David I. and Pierpont, Ross Z..... 119

Testosterone, Effect of, on the Blood Pressure and Tissues of Rats. Blackman, Samuel, S., Thomas, Caroline B. and Howard, John Eager..... 321
Thomas, Caroline B.: Experimental Hypertension from Section of Moderator Nerves..... 335
Thomas, Caroline B.: The Significance of Electrocardiographic Abnormalities in Young Adults..... 229
Thomas, Caroline B. (See Blackman, Samuel S.)..... 321
Trevett, Gordon I.: Experimental Studies on Sulfapyrazine in Mice..... 299
Tuberculosis Experimental, Immunization with the Vole Acid Fast Bacillus Against. Brooke, Wallace and Day, Robert..... 275

Vitamin C-Deficient Guinea Pigs, The Pathology of Staphylococcus Abscesses in. Meyer, Eugene and Meyer, Mary B..... 98
Vole Acid Fast Bacillus, Immunization with the, Against Experimental Tuberculosis. Brooke, Wallace and Day, Robert..... 275

White, Virginia (See Najjar, Victor A.)..... 378

FOR ORAL USE

There has long been a real need for a potent, mercurial diuretic compound which would be effective by mouth. Such a preparation serves not only as an adjunct to parenteral therapy but is very useful when injections can not be given.

After the oral administration of Salyrgan-Theophylline tablets a satisfactory diuretic response is obtained in a high percentage of cases. However, the results after intravenous or intramuscular injection of Salyrgan-Theophylline solution are more consistent.

Salyrgan-Theophylline is supplied in two forms:

Tablets (enteric coated) in bottles of 25, 100 and 500. Each tablet contains 0.08 Gm. Salyrgan and 0.04 Gm. theophylline.

Solution in ampuls of 1 cc., boxes of 5, 25 and 100; ampuls of 2 cc., boxes of 10, 25 and 100.

Write for literature

SALYRGAN-THEOPHYLLINE

"Salyrgan," trademark Reg. U. S. Pat. Off. & Canada

Brand of MERSALYL with THEOPHYLLINE

WINTHROP CHEMICAL COMPANY, INC.

Pharmaceuticals of merit for the physician

NEW YORK 13, N. Y. WINDSOR, ONT.

COPYRIGHT, 1943
MEAD JOHNSON & CO.
EVANSVILLE, IND. U.S.A.
PABLUM
PABENA

COPYRIGHT, 1943
MEAD JOHNSON & CO.